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LIFE HISTORY OF AGAMERMIS DECAUDATA, A NEMATODE PARASITE OF GRASSHOPPERS AND OTHER INSECTS [1]

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INTRODUCTION

Of the various nematodes that parasitize insects, those belonging to the family Mermithidae probably occur in the greatest variety of these hosts. As the presence of a mermithid parasite usually has a marked influence on the development of an insect, frequently retarding or modifying growth, usually inducing sterility, and finally resulting in death, these nematodes are of considerable interest from an economic standpoint. Reports from various parts of the world indicate that mermithids are an important factor in holding certain pestiferous insects in check, at least in some localities. As mermithids are usually long, slender animals they are sometimes referred to as "hairworms." Unfortunately this term has also been used for members of the Gordiidae, a group of animals whose resemblance to mermithids is largely superficial and with which they should not be confused.

In spite of the fact that mermithids have long been known to zoologists and numerous references to them are found in zoological literature, information regarding them is rather meager. In some instances writers have merely reported their occurrence and given a brief morphological description which is frequently so inadequate as to leave the reader in doubt whether the organism in question was a mermithid or a gordiid. However, numerous contributions, including the papers by Rauther (*13*)[2], Hagmeier (*9*), and Steiner (*16*, *17*, *18*, *19*), have contributed considerable information pertaining to the morphology and taxonomy of the group. In regard to life histories our knowledge is much less satisfactory. Although studies on this phase of the subject were made as early as 1853 by Von Siebold (*15*) and Meissner (*12*) and later by Corti (*5*), it was not until 1905 that Kohn (*11*), working with *Paramermis contorta* (Linstow, 1889) Kohn, 1905, a parasite of *Chironomus* larvae, presented the first comprehensive life-history study. Further observations on this species were made by Comas (*4*).

Judging from available information, it appears that the typical mermithid life cycle consists of a parasitic larval development in the

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[2] Reference is made by number (italic) to Literature Cited, p. 197.

body cavity of the host, followed by a free-living adult stage usually passed in the soil or in mud and sand underlying streams and lakes. *Tetradonema plicans* Cobb, 1919, which infests the larvae of *Sciara coprophila* Lintner, is evidently an exception, as it apparently passes its entire life as a parasite (*10*). This should probably be regarded as a specialized type of life cycle wherein the free-living stage has been eliminated.

In the north-central and northeastern parts of the United States, over an area extending southward to northern Virginia and westward to eastern Nebraska, the more common species of grasshoppers harbor mermithid parasites which consist largely of two species, *Agamermis decaudata* Cobb, Steiner, and Christie, 1923 (*3*), and *Mermis subnigrescens* Cobb, 1926. The evident economic significance of these parasites of grasshoppers has been noted by several investigators. I. G. McGrue, of Audubon, Minn., is reported by Riley (*14*) as asserting that "in that part of the country the hairworms destroyed as many locusts in 1875 as did any other enemy." Writing in 1900, Forbes (*7, p. 129*) makes the following comment:

> Long thread-like, milk-white hair-worms (*Mermis*) are often found in the abdomens of grasshoppers, living there as internal parasites, and escaping after maturity to enter the earth, where they pass the winter, pair, and produce myriads of eggs the following spring. The young from these infest the grasshoppers of the year and assist greatly in the reduction of any excess of numbers.

In August 1908 a grasshopper outbreak at Columbia Cross Roads, Pa., was investigated by J. A. Hyslop, of the Bureau of Entomology, United States Department of Agriculture. When specimens of *Melanoplus femur-rubrum* DeGeer, the species responsible for the outbreak, were dissected, cream-white worms, about 3 or 4 inches in length, were taken from the bodies of the insects. In one case 12 worms were taken from a female *M. femur-rubrum*, 10 of which were 3 inches long. Hyslop observed that "the infested grasshoppers act sluggish" and that "the genital organs (ovaries and testes) were always consumed where worms were present."[3]

Glaser and Wilcox (*8*), while investigating a grasshopper epidemic near Dummerston Station, Vt., in 1917, noted a high mortality among these insects. This mortality was due to a mermithid parasite which infested nearly 30 percent of all the grasshoppers in that locality at the time, *Melanoplus atlanis* Riley and *M. bivittatus* Say being the most common species. Between 1923 and 1925 there occurred in Door County, Wis., a grasshopper outbreak of considerable severity, the principal species being *Camnula pellucida* Scudd. The writer visited this region during August 1925 and found the grasshoppers heavily infested with mermithid parasites. There is little doubt that these parasites were an important factor in terminating the plague.

The parasite involved in the grasshopper outbreak in Door County, Wis., was *Mermis subnigrescens* and that found by Glaser and Wilcox at Dummerston Station, Vt., was probably the same species. The parasite found by Hyslop at Columbia Cross Roads, Pa., was probably *Agamermis decaudata*, a species which is abundant in northeastern Virginia. It is the species with which the present paper deals.

For convenience and clarity in description, the life cycle of *Agamermis decaudata* is divided into five stages, as follows: (1) The

[3] HYSLOP, J. A. Unpublished note

embryonic and ovic larval stage, which includes the development of the embryo from the time the egg is deposited until the larva emerges; (2) the preparasitic larval stage, which includes the period between hatching and the entrance of the larva into a host; (3) the parasitic larval stage, which includes the development within the host; (4) the postparasitic larval stage, which extends from the time the larva emerges from the host until it undergoes its final molt; and (5) the adult stage.

EMBRYONIC AND OVIC LARVAL STAGE

DEPOSITION, STRUCTURE, AND SIZE OF THE EGGS

The adults of *Agamermis decaudata* are found coiled within small cavities in the soil, usually about 5 to 15 cm below the surface. Here eggs are deposited and undergo embryonic development, becoming plastered over the parent nemas and over the walls of their earthen cavities. It is estimated that an average female during her life will deposit about 10,000 eggs.

The egg when first deposited (fig. 1, *A*) is somewhat discoidal, having the form of a sphere considerably flattened on opposite poles. As ordinarily seen under the microscope it presents a circular outline measuring, on an average, about 160μ in diameter. When viewed on edge it is ellipsoidal in outline, the length of the short axis being about two-thirds that of the long axis. The protoplasmic mass is surrounded by a thin membrane. Outside of this, in the case of *Hexamermis albicans* (Siebold, 1848) Steiner, 1924, Meissner (*12*) reported the existence of a colorless transparent layer composed of a semifluid substance which he called the albuminous layer (Eiweisschicht). Enclosing the whole is an outer membrane or "shell", 1.5μ to 2μ thick and of a pale amber or straw color. The color, at first very faint, darkens as the egg develops.

Adult females of *Agamermis decaudata* collected by the writer varied in length from 3 to 46.5 cm. It was noted that eggs deposited by the smaller females averaged about 120μ in diameter (measured on the longer axis) as against an average of 163μ for those deposited by larger females. Results from a study of the length of females and the size of eggs deposited by them are shown in table 1.

TABLE 1.—*Relation between length of female Agamermis decaudata and size of eggs*

Length of female (cm)	Diameter of eggs			Length of female (cm)	Diameter of eggs		
	Minimum	Maximum	Average		Minimum	Maximum	Average
	μ	μ	μ		μ	μ	μ
3.0	121	130	126.9	25.0	159	174	166.3
3.2	110	118	113.0	27.0	150	170	163.2
3.5	110	118	114.0	28.0	160	178	167.8
4.5	106	120	110.4	28.0	164	172	168.9
5.0	110	125	116.8	30.0	150	179	161.3
6.0	125	133	129.0	31.0	155	176	166.5
6.0	123	127	125.4	33.0	147	164	156.0
8.0	103	137	119.2	34.0	144	180	160.1
9.5	120	137	129.1	35.0	146	160	153.6
12.0	115	141	125.2	36.0	144	172	156.0
15.0	154	182	169.7	45.0	162	168	166.0
18.0	154	176	166.9	46.5	164	170	167.5
24.0	141	164	153.5				

The data in table 1 show that the size of the eggs deposited does not increase gradually as the length of the female increases. By comparing the average measurements of the eggs deposited by each female it will be observed that those deposited by females 3 to 12 cm long comprise one group while those deposited by females 15 to

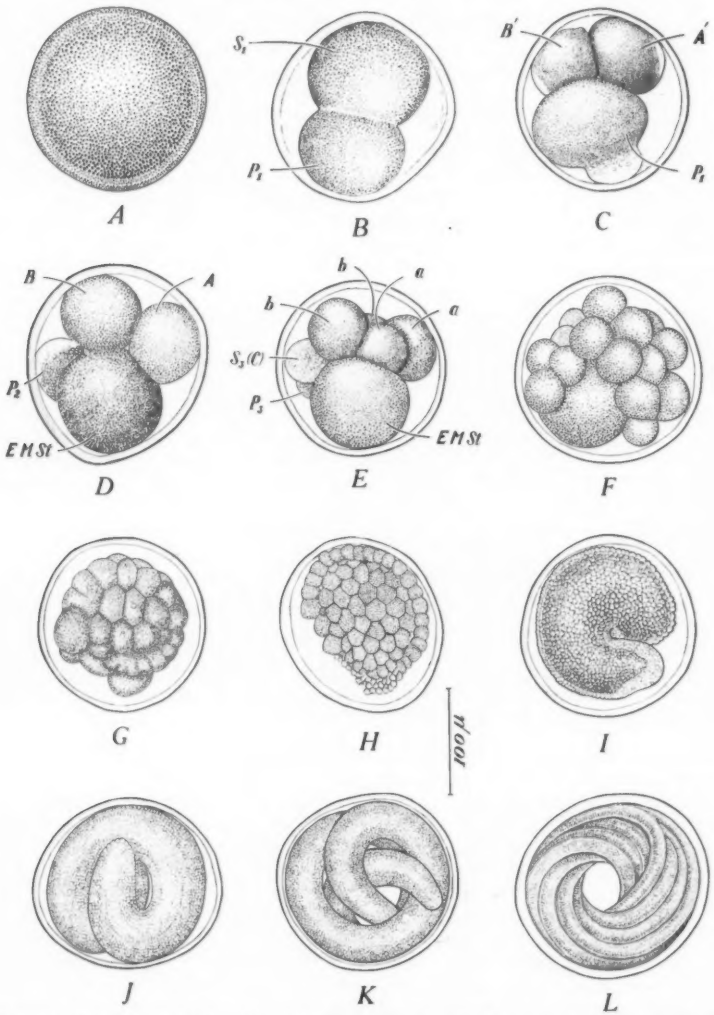

FIGURE 1.—Egg of *Agamermis decaudata*: *A*, At time of deposition; *B*, after about 24 hours; *C*, after about 30 hours; *D*, after 36 to 40 hours; *E*, after 4 to 5 days; *F*, after 6 to 7 days; *G*, after 8 to 9 days; *H*, after 10 to 11 days; *I*, after 13 to 15 days; *J*, after 16 to 18 days; *K*, after 20 to 24 days; *L*, after 35 to 40 days. S_1, First somatic stem cell; P_1, undifferentiated cell; *A'*, anterior primary ectoderm cell; *B'*, dorsal ectoderm cell; P_2, second undifferentiated cell; *EMSt*, endoderm-mesoderm-stomodaeum cell; $S_2(C)$, posterior dorsal ectoderm stem cell; P_3, undifferentiated stem cell; *a*, *a*, *b*, *b*, cells of primary ectoderm.

46.5 cm long comprise another group. If the variations in the size of the eggs be represented by a curve, this curve will be "two-peaked" with but a slight overlapping at the base. At first one is inclined to suspect the existence of two species. Such information as we possess, however, indicates that there is but one species, although the point is not yet settled. The matter will be discussed further in connection with the preparasitic larva.

CLEAVAGE

During the first 10 to 12 hours after deposition, the central protoplasmic mass contracts slightly until there exists between it and the outer shell a clear space 30μ to 35μ in thickness. The first cleavage is perpendicular to the flattened poles of the egg and results in two cells slightly unequal in size. This division is usually complete at the end of 24 to 30 hours after the egg is deposited. The larger of the two blastomeres (fig. 1, *B*, S_1), is the first to divide, resulting in two cells about equal in size. The remaining blastomere of the first pair (fig. 1, *C*, P_1), now divides. On the side opposite the two small blastomeres now present (A' and B'), a projection of protoplasm is pushed out and is eventually split off. The cell thus formed (fig. 1, *D*, P_2), is smaller than any of the others and differs from them in appearance, being composed of a somewhat clearer protoplasm. This cleavage is usually complete at the end of about 40 hours and leaves the egg in a four-celled stage. Cell P_2 divides, resulting in two blastomeres, P_3 and S_3 (*C*), each of which again divides. In the meantime the derivatives of the larger of the first two blastomeres (A' and B') continue in their cleavage, producing a cluster of cells which tends to grow over and envelop the large blastomere (*EMSt*). This latter undergoes 2 or 3 cleavages before it is completely hidden, each division resulting in daughter cells about equal in size. At the end of 8 to 9 days the young embryo has developed to the stage shown in figure 1, *G*. In 16 to 18 days an embryo of about one and a half coils (fig. 1, *J*) is produced, and after this the number of coils increases rapidly. At first there is a tendency for the coils to assume an irregular, crisscross arrangement, and 20- to 30-day embryos are likely to be in this condition; but after about 30 days the coils arrange themselves near the periphery of the disk (fig. 1, *L*).

DEVELOPMENT OF THE STYLET

In the head region of a 20-day ovic larva the outline of the developing esophagus (fig. 2, *A*, *oe*) can usually be seen. Posteriorly it becomes increasingly indistinct, disappearing about 40μ from the anterior end. The rounded anterior contour is more difficult to see than the walls which bound it laterally. About 16μ from the anterior end of the larva the esophagus is constricted, thus forming a differentiated anterior part (fig. 2, *A*, *oe ant*). Back of the constriction the esophagus has about the same diameter as the part anterior to the constriction. Near the center of the anterior part the primordium of the odontium or tooth can be seen (fig. 2, *A*, *vesic*). It is a pyriform vesicle about 8μ long by 3μ wide, and in a living specimen appears somewhat lighter in color than the surrounding tissue.

In a 25-day ovic larva the esophagus is still a prominent feature, the anterior part having enlarged to a diameter four-fifths as great

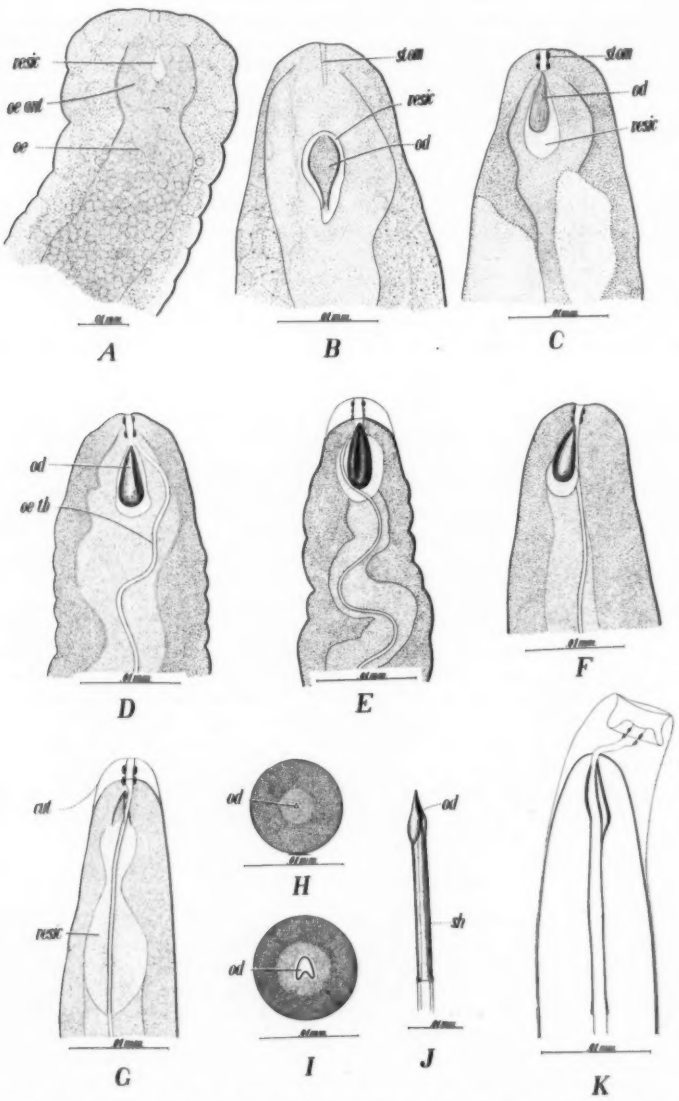

FIGURE 2.—Development of stylet in *Agamermis decaudata*: *A*, 20-day larva, lateral view; *B*, 25-day larva, lateral view; *C*, 28-day larva, lateral view; *D*, 30-day larva, lateral view; *E*, 35-day larva, dorsal view; *F*, 40-day larva, lateral view; *G*, 45-day larva, lateral view; *H*, 30-day larva, cross section through tip of odontostyl "head"; *I*, 30-day larva, cross section through middle of odontostyl "head"; *J*, fully developed odontostyl, semidiagrammatic; *K*, molt at end of 55 days, lateral view. *stom*, Stoma; *cut*, cuticle; *oe*, esophagus; *oe ant*, anterior enlargement of esophagus; *oe th*, esophageal tube; *od*, odontium; *hd*, "head" of odontostyl; *sh*, "shaft" of odontostyl; *vesic*, vesicle.

as the diameter of the larva in that region. The vesicle (fig. 2, *B*, *vesic*) is about twice as long as wide and has a diameter one-third as great as the diameter of the esophageal enlargement. In the center of the vesicle and nearly filling it is a spindle-shaped mass of rather dense substance, bounded laterally by heavily cuticularized walls (fig. 2, *B*, *od*).

In an average 28-day ovic larva the esophagus and the anterior esophageal enlargement are both more clearly defined than in preceding stages. The developing odontium (fig. 2, *C*, *od*) now has the shape of an elongated cone with a pointed anterior end and a hemispherical base. It has moved slightly anteriad and is enclosed in the vesicle, which is about one-third larger and may somewhat resemble it in shape. The wall of the stoma is reinforced by 2 or sometimes 3 hooplike (?) thickenings which when seen in lateral aspect present the appearance shown in figure 2, *C*. The tubular lumen of the esophagus may sometimes be seen at about this stage of development.

The average 30-day ovic larva shows few changes from a 28-day larva. On the ventral side of the developing odontium (fig. 2, *D*, *od*), a groove extends from near the point to near the base. This groove may best be understood by studying a front view of the head, where the odontium appears crescent-shaped in optical cross section (fig. 2, *H*, *L*, *od*). A tortuous, heavy-walled tube now occurs within the esophagus, extending past the vesicle and terminating near the base of the stoma, with which it may possibly communicate, although the opening was not seen.

In a 40-day ovic larva (fig. 2, *F*) the walls of the esophagus are more difficult to observe, and as the larva develops they become increasingly obscure. The stoma now communicates directly with the esophageal tube, which runs straight back without the windings characteristic of earlier stages. The developing odontium, which up to this time has occupied a more or less axial position, now moves toward the dorsal side, the groove lying adjacent to the esophageal tube. The vesicle often encompasses only the base of the odontium and in some cases seems to have disappeared. At about this time a second vesicle (fig. 2, *G*, *vesic*) is formed, oblong in shape and encompassing that portion of the esophageal tube which is destined to form the shaft of the stylet. The wall of this part of the tube now becomes thicker. At the end of about 50 days the stylet (fig. 2, *J*, *K*) is completely formed. When the odontium is united with the shaft the anterior extremity of the esophageal tube lies in a groove of the odontium (fig. 2, *G*), with which it is eventually joined. The odontium, therefore, acts as a reinforcement which is wrapped around and united with the anterior end of the shaft.

For a stylet formed from a modified tooth or odontium or from an odontium together with a portion of the esophageal tube, as in the present instance, the term "odontostyl" is proposed. Such stylets also occur in the genus *Dorylaimus* and related genera. For a stylet formed through a modification of the entire stoma or buccal cavity the term "*stomatostyl*" is proposed. Such stylets occur in the genera *Anguillulina* (=*Tylenchus*) and *Aphelenchoides*.

TWENTY-DAY OVIC LARVA

At the end of 20 days the larva usually forms about two and one-half loops within the egg shell (fig. 1, *K*). The anterior end of such a larva, for a distance of 30μ or 40μ, is filled with a comparatively clear, finely granular tissue in which distinct cell walls were not seen. As already noted, it is in this clear anterior region that the developing esophagus is visible. Back of this region the body is filled with spherical globules which vary from 1.5μ to 3μ in diameter. In situ these globules tend to be polygonal because of the pressure of one upon another, but when broken from the body they become spherical. They are probably fat or oil globules and contain a clear nongranular substance that darkens with osmic acid.

THIRTY-DAY OVIC LARVA

At the end of 30 days the larva (fig. 3) has attained a length nearly equal to that of the preparasitic larva. There is a cylindrical stoma 3μ to 4μ long by 2μ wide, its wall reinforced by the hooplike (?) thickenings already mentioned. Whether these extend entirely around the stoma was not determined, but from a study of the molted lining they seemed to be more strongly developed dorsally and ventrally. In lateral view they appear as 2 or 3 pairs of dark spots (fig. 2, *G*). As the diameter of the esophageal tube is less than that of the stoma, the latter is narrowed somewhat where it joins the former. This reduction in width takes place on the dorsal side, causing in profile view a slightly asymmetrical appearance.

The anterior portion of the esophagus is well differentiated; it is a subcylindrical structure 210μ [4] in length and possesses three noticeable swellings or enlargements. The first (fig. 3, *oe enl* (*1*)) is at the anterior end and contains the developing odontium. Posteriorly the diameter decreases rather gradually to about 6μ to 7μ. The second enlargement (fig. 3, *oe enl* (*2*)) is slightly anterior to the nerve ring. Here the width increases gradually to about 12μ, then decreases to 4μ or 5μ. This width is retained for a distance of about 80μ and then again increases to 10μ or 12μ to form the posterior enlargement or bulb (fig. 3, *blb*). The bulb is partly obscured by granules and globules, which fill the body from this point posteriad.

A distinct esophageal tube, 0.5μ to 1μ in diameter, occurs within the esophagus throughout this anterior part. Embedded in the dorsal wall of the esophageal enlargement at the nerve ring and with a diameter two-thirds as great as that of the enlargement itself, is a large, clavate cell (fig. 3, *cl*). The wider end of the cell is directed cephalad and the narrower end extends through the nerve ring. A spherical nucleus is located near the anterior end of the cell. As seen in lateral aspect this cell is 30μ long by 8μ wide. Extending caudad from the esophageal bulb for a distance of 200μ, the posterior portion of the esophagus (fig. 3, *post oe*) can be faintly seen. It is nearly cylindrical, has a diameter two-thirds as great as the corresponding body diameter, and is packed with granules or globules that are smaller and different in appearance from those that surround and partly obscure it. This structure probably includes not only the esophagus proper but the primordia of the glands and other organs later associated with it, although they cannot now be distinguished.

[4] Measurements given in the morphological descriptions were derived from 3 or 4 specimens selected as being of about average size.

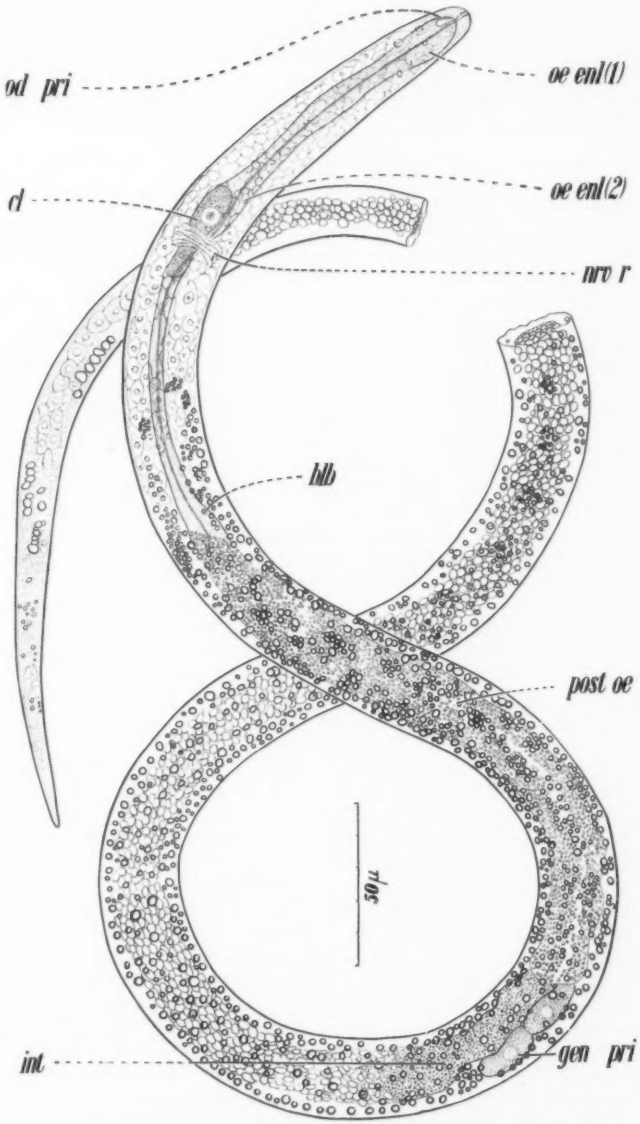

FIGURE 3.—Ovic larva of *Agamermis decaudata* 30 days after deposition of egg: *blb*, Bulblike enlargement of esophagus; *cl*, cell (?); *gen pri*, genital primordium; *int*, intestine; *od pri*, primordium of "head" of odontostyl; *oe enl (1)*, anterior enlargement of esophagus; *oe enl (2)*, middle enlargement of esophagus; *nrv r*, nerve ring; *post oe*, posterior part of esophagus.

Back of the esophagus the developing intestine (fig. 3, *int*), 50μ long, is faintly visible. On the ventral side, at the junction of the intestine and esophagus, is located the primordium of the reproductive system (fig. 3, *gen pri*). It is apparently composed of two cells, each of which is 15μ to 20μ long by 6μ to 8μ wide. Caudad from the posterior end of the intestine the body is filled with a continuous cylindrical mass of closely packed granules and globules.

FORTY-DAY OVIC LARVA

At the end of 40 days the ovic larva (fig. 4) is very similar in structure to the preparasitic larva. The esophagus is composed of an anterior and posterior part. The anterior part is possibly homologous to the corpus and the posterior part to the isthmus and bulb as found in certain other nematodes. In the anterior part the swellings noted in the 30-day larva have disappeared, and there remains a cylindrical structure 4μ in diameter containing a conspicuous tube 1.5μ to 2μ in diameter. At about 220μ from the cephalic end of the animal the esophagus enlarges to a width of 10μ and at the point where this enlargement begins the character of the lumen is abruptly altered. This change is from a tube more or less circular in cross section to one with a triradiate cross section and marks the junction of the anterior and posterior parts of the esophagus. The posterior part has a length of about 300μ. Its anterior end has the form of a bulblike swelling (fig. 4, *blb*) 35μ long by 10μ wide. The remaining position, 245μ in length, while perhaps structurally similar to the bulblike swelling, is greatly modified in shape by the presence of three large glands. The largest of these glands (fig. 4, *subd oe gl*) is right subdorsal in position. It first appears in about 35 days as a subcylindrical structure extending anteriad from the base of the esophagus about two-thirds the distance to the bulblike enlargement. In a 40-day larva it has increased considerably in diameter, extends farther anteriad, and often appears to have a branched anterior end. It is filled with closely packed, nearly spherical globules averaging about 3μ in diameter. At its anterior end a mass of similar globules often forms a separate body, the size and shape of which differ in different specimens. On the left subventral side there are eventually developed two smaller glands. These, however, are usually not discernible at this stage and will be discussed in connection with the preparasitic larva. The excretory pore is opposite the nerve ring. The pore itself was not seen, but the duct leading to it can usually be detected.

The genital primordium (fig. 4, *gen pri*) remains about the same as in a 30-day larva. The intestine has increased slightly in length, but otherwise it has changed little. The granules at the posterior end of the intestine are arranged in transverse rows (fig. 4, *nd*), preparatory to the formation of the node, a structure to be described later.

When a 30- to 40-day larva is removed from the egg sometimes the cuticle is seen separating from the body at the two extremities. This is the beginning of the first molt. As molting progresses the cuticle is frequently invaginated at the anterior end because of the lining of the esophagus (fig. 2, *K*). The cuticle finally breaks at some point on the body, and the anterior portion is pushed off over the head and the posterior portion over the tail. In an egg containing an almost

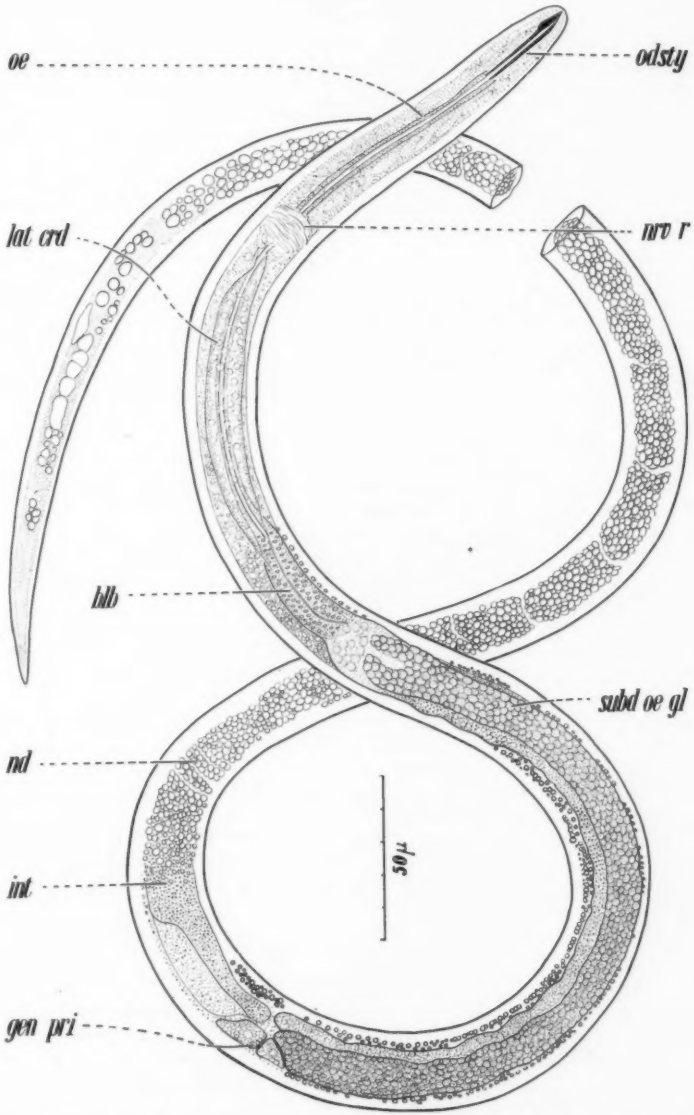

FIGURE 4.—Ovic larva of *Agamermis decaudata* 40 days after deposition of egg. *blb*, Bulblike enlargement of esophagus; *gen pri*, genital primordium; *int*, intestine; *lat crd*, lateral cord; *nd*, node; *nrv r*, nerve ring; *oe*, esophagus; *odsty*, odontostyl; *subd oe gl*, subdorsal esophageal gland.

fully developed ovic larva the cast cuticle can usually be seen wound into a ball and located near the center of the egg. At a temperature of 12° to 25° C., molting begins within 1 to 2 months. The lining of the stoma and of the esophageal tube, as well as the rings (?) reinforcing the wall of the former, are shed with the old cuticle. Sometimes the outer cuticle breaks from the lining of the esophageal tube and stoma, and the latter is left protruding from the mouth. In one instance of this kind the ejection of this lining was completed while the specimen was under observation. The length of the ejected lining was equal to the distance from the anterior end to a point opposite the genital primordium, indicating that this entire region of the alimentary tract is cuticular-lined stomodaeum.

TIME OF HATCHING

At the beginning of winter each adult female is surrounded by the eggs produced by her during the preceding summer. All stages of development are represented, many eggs containing fully developed ovic larvae. While an occasional egg will hatch during the winter, hatching, for the most part, is suppressed until the advent of warm spring weather.

On November 27, 1924, three females were dug from the soil in such a manner that the eggs surrounding them were not disturbed. Each female was placed in a container devised for the purpose, and reburied. When first examined on February 4, 1925, some of the eggs had already hatched, and in every container a few larvae were found in the soil near the eggs. On April 22 larvae could not be found around any of the egg masses, but on May 5 three active larvae were seen in one of the containers. On May 19, active larvae were fairly numerous in all three containers. On June 2, active larvae were present but not so numerous as on May 19. On June 21, active larvae were again fairly numerous. However, the egg masses had not been noticeably depleted and the number that had hatched up to this time represented but a very small percentage of the total number of eggs present on November 27. On July 12, the soil in one of the containers was teeming with active larvae and nearly all of the eggs had hatched. In the other two containers the eggs had all hatched and the larvae had apparently migrated, for none was found.

From the foregoing observations it appears that, in the region of Falls Church, Va., in spite of a limited amount of hatching during winter and early spring, the greater part of the larvae emerge between late June and the middle of July.

PREPARASITIC LARVAL STAGE

STRUCTURE OF THE PREPARASITIC LARVA

Ovic larvae, when kept at room temperature, appear to be fully developed within 50 to 60 days. However, they do not leave the egg immediately. In nature, as already noted, a larva which becomes fully developed during late summer will ordinarily remain within the egg until the following spring.

Preparasitic larvae (fig. 5) vary in length from 4.42 to 5.59 mm, the majority being from 5 to 5.10 mm. This does not include individuals hatched from the smaller type of eggs previously referred to, the taxonomic status of which will be discussed later. The width of the

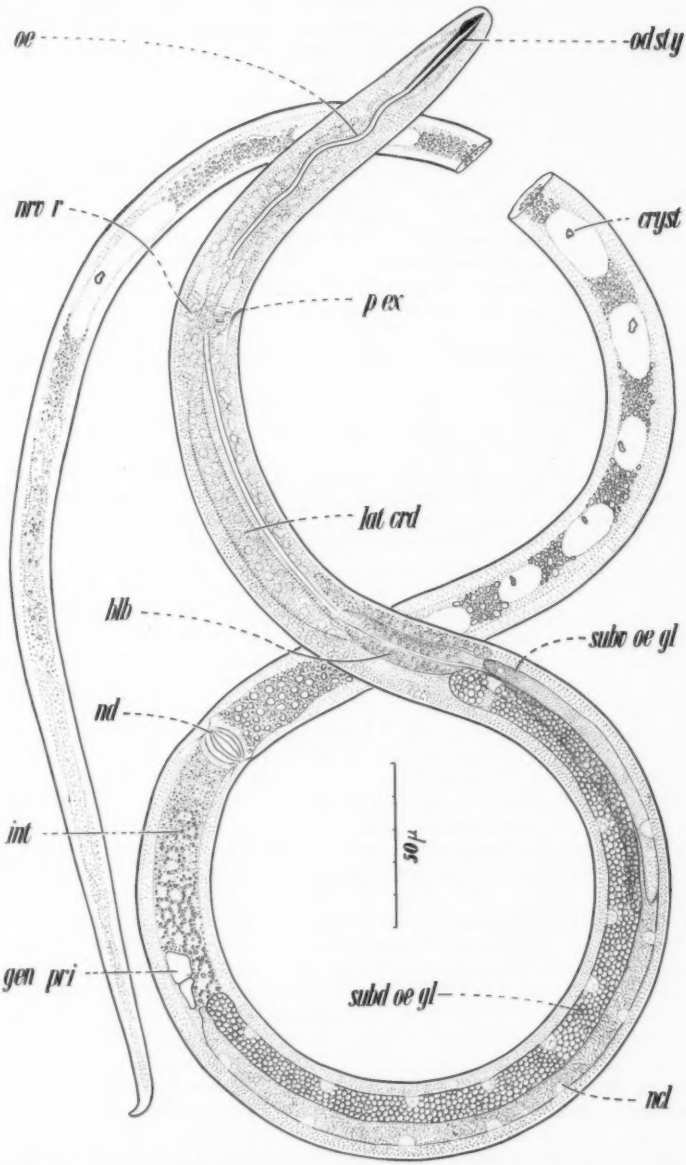

FIGURE 5.—Preparasitic larva of *Agamermis decaudata: blb*, Bulblike enlargement of esophagus; *cryst*, crystals, probably of excretory materials; *gen pri*, genital primordium; *int*, intestine; *lat crd*, lateral cord; *ncl*, nuclei of primordia of stichocytes; *nrv r*, nerve ring; *oe*, esophagus; *nd*, node; *odsty*, odontostyl; *p ex*, excretory pore; *subd oe gl*, subdorsal esophageal gland; *subv oe gl*, subventral esophageal gland.

body is as follows: At the base of the spear, 16μ to 17μ; at the nerve ring, 20μ to 22μ; at the esophageal bulb, 22μ to 24μ; at the base of the esophagus (region of the genital primordium), 23μ to 25μ; slightly posterior to the node, 20μ to 22μ. The body is divided into an anterior region, which contains the principal organs, and a posterior region, which is a food-storage region and a propelling organ. These two body regions are separated by the node (fig. 5, *nd*), where an automatic amputation takes place when the larva enters its host. The cuticle is 1.5μ to 2μ thick and appears smooth. If it is transversely striated the striae are exceedingly difficult of resolution. The head is rounded and bears six small papillae. The amphidial openings are situated 6.5μ from the anterior extremity. In carmine-stained specimens the internal amphidial structure is conspicuous, extending posteriad to a point 4μ distant, where it seems to end abruptly, the stain not having penetrated farther. The odontostyl (fig. 5, *odsty*) is from 33μ to 37μ long and is composed of an enlarged subconical pointed head 8μ long by 3μ wide and a posterior shaft 2μ wide and very slightly expanded at its base. The entire structure is hollow, with the anterior opening on the ventral side. The odontostyl is surrounded by well-developed muscles.

The anterior portion of the esophagus is about the same as that already described for a 45-day ovic larva. Transverse sections through the cuticularized esophageal tube show that a thin layer of tissue surrounds the tube, although the total diameter of the esophagus in this region does not exceed 4μ. While in general the tube is circular, in transverse section its shape is often suggestive of a triradiate origin. In parasitic larvae the triradiate character of this part of the lumen is sometimes very apparent.

About 245μ from the anterior end of the body the esophagus widens to form a bulblike enlargement (fig. 5, *blb*) 10μ in diameter, which possesses distinct radiating muscles. As previously noted, the lumen changes abruptly in character at the anterior end of the bulb. The posterior portion of the esophagus is partly obscured by the esophageal glands, which extend nearly to the posterior margin of the bulb. The anterior end of the largest gland (fig. 5, *subd oe gl*) lies about 35μ behind the anterior end of the bulb, and the gland extends caudad the entire length of the esophagus, a distance of about 270μ. It is right subdorsal in position and is packed with clear, spherical globules 1μ to 1.5μ in diameter. There is a region at its anterior end which is somewhat different in appearance and either contains a small group of globules somewhat separated from the others, or lacks globules and is filled with a homogeneous nongranular substance. The two smaller esophageal glands (fig. 5, *subv oe gl*) are left subventral in position and are filled with a homogeneous substance of a granular appearance. Their anterior ends lie slightly behind the anterior ends of the subdorsal gland. They extend caudad 90μ to 100μ and at their widest point have a diameter of 6μ to 8μ. A nucleus is situated near the anterior end of each. These glands communicate with the lumen of the esophagus through pores. The esophagus proper lies between these glands and is slightly compressed by them. In cross section it appears as a somewhat triangular structure, almost axial in position, with the lumen near its center. Sixteen rather conspicuous nuclei are found in this region of the body. Posteriorly they have a tendency to be arranged in pairs, one on each side of

the esophagus, although their position varies somewhat in different specimens. They are spaced more or less equidistant and the first pair lies slightly in front of the posterior end of the subventral glands. They are nuclei in the primordia of the so-called "stichocytes."

The intestine (fig. 5, *int*) is 80μ to 90μ long and extends from the base of the esophagus to the node. It is without a distinct lumen and contains two kinds of granules arranged in a loose network. The smaller and more numerous of these granules do not stain intra-vitam with neutral red, while the larger granules stain promptly. In the anterior two-thirds of the intestine, cell walls could not be seen, but in the posterior part definite transverse walls are present, becoming more conspicuous near the node. A varying number of prominent, slightly concave, transverse septa mark the position of the node. One or sometimes several of the most posterior septa have their convex surface posterior, while the others have their convex surface anterior, and between these there is a small clear space. It is at this point that the amputation of the caudal portion of the body takes place; the septa remaining on the body proper form a plug, which prevents the body contents from exuding. The part posterior to the node constitutes about 80 percent of the total length of the larva. The width back of the node, 20μ to 22μ, is slightly less than that in front of the node, and the caudal portion retains this width throughout two-thirds of its length. It then gradually tapers posteriorly, ending in a pointed terminus, which may sometimes be curved at the tip. Except for a terminal portion of about 200μ, it is filled with a moniliform series of about 50 cells. Each cell has a central nucleus surrounded by a network of protoplasm containing spherical refractive globules up to 5μ in diameter and smaller granules of 1μ or less. The cells are separated by a clear space containing 1, or occasionally 2, refractive, irregular-shaped crystals.

In a 25- to 30-day ovic larva the entire caudal portion, except for a short terminal region, is packed with spherical globules forming a continuous cylinder. In about 40 to 45 days these globules become so arranged as to form short cylindrical "segments", each of which is separated from those adjacent by a narrow space. Back of the node these segments are 20μ to 30μ long and increase in length posteriad until they become 70μ to 80μ long near the posterior extremity. As the larva develops, the clear spaces separating the segments increase in size, and at the time of hatching each usually contains a small crystal (fig. 5, *cryst*). As the larva becomes older the globules decrease in number and after a few days have nearly all disappeared. At the same time the clear spaces grow larger, and there is a slight increase in the size of the crystals. These globules, which do not stain intra-vitam with neutral red, are probably reserve material which is gradually utilized. The contents of the clear spaces, including the crystals, are interpreted as waste products of metabolism. Among the globules are smaller granules which stain deeply with neutral red, in this respect resembling similar granules in the intestine.

MIGRATION TO THE HOST

After hatching, the preparasitic larvae must migrate to the surface of the soil to locate their insect hosts. Although *Melanoplus femur-rubrum*, like many Acridiidae, deposit their eggs in the soil, the writer

regards it as improbable that nymphs become infested before leaving the soil. Such a possibility is precluded in the case of *Orchelimum vulgare* Harris and many other tettigoniids by the fact that eggs are deposited in the stems of plants. Observations made both on grasshoppers in the field and on those hatched and reared in the laboratory indicate that nymphs of *M. femur-rubrum* do not often come into actual contact with the soil, but tend to feed and rest on grass and other vegetation. Observations made at night revealed nymphs of this and other species of grasshopper resting on the plants and not on the ground. It appears necessary that the preparasitic larvae climb the grass and other vegetation in order to reach their hosts. As drying is fatal, this climbing must be done either at night or early in the morning while the grass is wet with dew, or during rainy weather. That the larvae will climb a moist surface has been demonstrated in the laboratory, where they were found ascending the glass sides of an insect cage.

METHOD OF ENTERING THE HOST

Although the writer has never observed the entrance of the preparasitic larva into the host in nature, experimental infestation of insects in the laboratory is easily accomplished. As the eggs of both *Agamermis decaudata* and *Melanoplus femur-rubrum* hatch readily when brought into the laboratory, an abundance of young individuals of both host and parasite may be had throughout the winter for experimental purposes. To infest a grasshopper nymph with *A. decaudata* the following method was employed and proved satisfactory.

A glass slide was placed on a turntable and with a small candle a wax ring (fig. 6, *a*) about one-fourth of an inch in diameter was run on. With a scalpel a narrow channel was made through this ring at two points (*b*, *c*), in which was laid a long hair. One end of the hair was sealed down with a drop of hot wax (*d*), which at the same time closed that channel. The nymph was wet by being shaken in a vial containing water. This not only rendered the nymph less active and easier to handle but facilitated the entrance of the larvae. With a moistened camel's-hair brush, the nymph was transferred to the slide and placed on its side within the ring. The hair was drawn across its body just back of the pronotum. The free end of the hair was then sealed down with a drop of wax, which at the same time closed the other channel. A nymph thus pinioned may be held securely for a long time without injury. The nymph should rest in a drop of water in which are placed active preparasitic larvae.

The larvae swim rapidly at first, moving in an apparently aimless way, often following the periphery of the drop, and are not noticeably attracted by the presence of the insect. After a few minutes some may be seen with their heads pushed against the nymph or inserted between the segments. Entrance does not necessarily follow this act as the larvae may move or be kicked away and resume their apparently aimless swimming. Soon, however, detached caudal ends will usually appear, and if the detached anterior ends cannot be seen it may be assumed that infestation has taken place. The activity of the nymph often breaks larvae at the node before they have had an opportunity to enter. The detached anterior ends continue active movement and have been seen attempting to bore their way into the nymph, but

hampered by the loss of their propelling mechanism, probably never succeed. The larvae appear to attack the nymph at any point without preference, and have on occasion been seen boring at the distal end

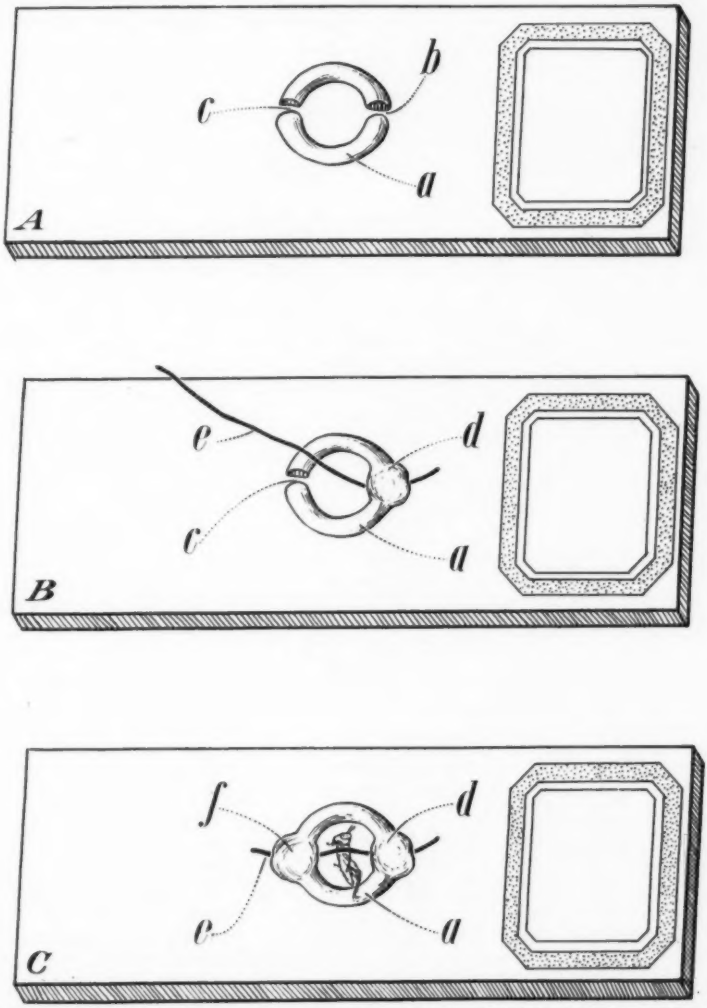

FIGURE 6.—Device with which grasshopper nymphs were experimentally infested: *A*, Glass slide with wax ring (*a*) divided at two points (*b*, *c*); *B*, wax ring (*a*) closed at one point by drop of wax (*d*) in which is embedded end of hair (*e*); *C*, wax ring (*a*) closed by second drop of wax (*f*), with nymph fastened in center by hair (*e*).

of the posterior femur. They seem able to penetrate more easily through the thorax, and probably the majority enter near this region. Despite the fact that the entire operation can be kept under the closest scrutiny, it is exceedingly difficult actually to see the larvae go in.

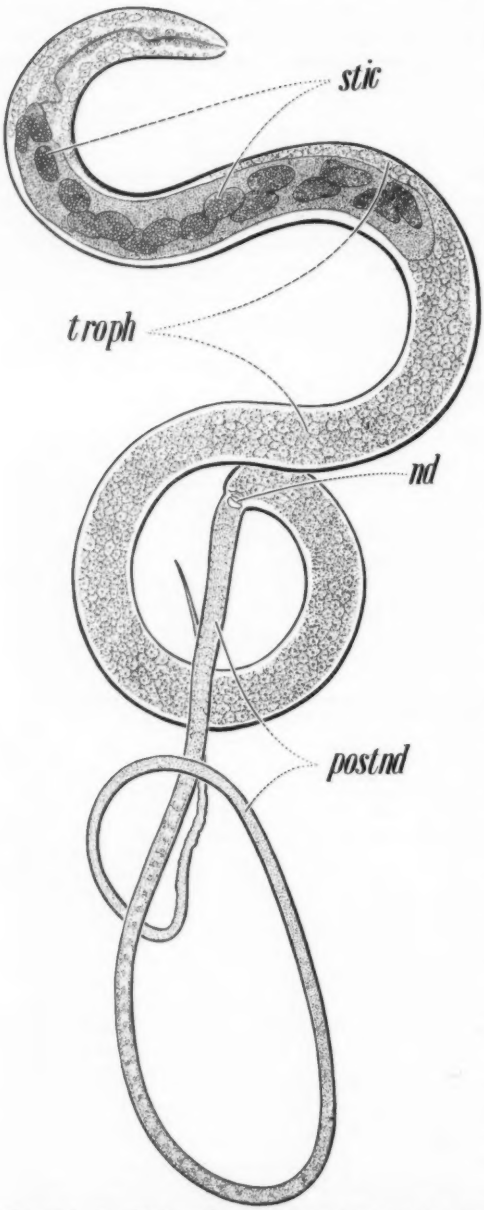

FIGURE 7.—Parasitic larva of *Agamermis decaudata*, about 6 to 8 days in the host, in which the postnodal portion of the body was not detached when the larva entered the host: *nd*, Node; *postnd*, postnodal region of body; *stic*, stichocytes; *troph*, trophosome (modified intestine).

Because of their minute size and the high magnification necessary to follow their movements carefully, they are lost from view immediately they come into contact with the nymph. In only a few cases, where light conditions were exceptionally favorable, were larvae seen in the process of entering. The time necessary for making the puncture varies considerably. Sometimes they penetrate within 1 to 2 minutes and at other times they bore for 5 to 10 minutes. Once the head is inserted they enter immediately.

At this time they usually break at the node, leaving the posterior portion of the body outside the host, although occasionally, the amputation does not take place and the posterior portion is carried in (fig. 7). Although the breaking at the node may usually be caused by the activity of the host, this is not necessarily true. In one experiment a 1-day-old nymph of *Melanoplus femur-rubrum* was pinioned on a slide and 10 active preparasitic larvae were placed around it. Except for some movement immediately after it was pinioned, the grasshopper remained absolutely quiet. In about 5 minutes 2 detached pos-

terior ends and 1 detached anterior end were seen; a few minutes later another detached anterior end was noticed, and finally a larva was seen actually breaking at the node. The breaking of this larva before the anterior end had penetrated the host could not have been due to any movement of the nymph.

By counting the larvae and the detached anterior and posterior ends that remain on the slide after the nymph is removed, it is possible to approximate with considerable accuracy the number of larvae that have entered. In 88 experiments in infesting first-instar nymphs of *Melanoplus femur-rubrum*, 519 larvae were used, of which 304, or 58 percent, succeeded in entering. These figures were secured by actual dissection of the hosts.

TWO TYPES OF LARVAE

A difference in the size of eggs deposited by females of different lengths has already been mentioned (p. 163). There is an even more pronounced difference in the size of the larvae that develop from these eggs. Preparasitic larvae from eggs of the smaller type range from 2 to 2.18 mm in length, the distance from the head to the node being 370μ to 410μ, and the distance from the node to the caudal extremity 1.64 to 1.77 mm. Preparasitic larvae from eggs of the larger type range from 4.42 to 5.57 mm in length, the distance from the head to the node being 610μ to 840μ and the distance from the node to the caudal extremity 3.84 to 4.81 mm.

Cobb, who studied these larvae in 1923, regarded the smaller type as probably a different species. The following is quoted from unpublished notes by this investigator:

> These larvae closely resemble those of *Agamermis decaudata* but with the following differences: They are much smaller; the node is differently constructed; there are about half as many trophocytes [5] (33); the tail end is hamate and there is a more decided difference in the diameters of the cephalic and caudal parts. In *A. decaudata* about 8 to 10 cells take part in building the node, while here apparently there is only one such cell, so that instead of presenting a roll-of-coin-like series of cells just in front of the node there is but a single, flattish, refractive cell (?) at the node. There is a difference also in the nodal contour below the subcuticle. The constriction is larger and more conspicuous than in *decaudata*, in which sometimes there is very little evidence of a constriction. Opposite the constriction in the present species is a single roll-of-coin-like cell; behind the constriction what may be called the protoplasmic portion of the interior of the nema expands, approaches closely to the cuticle, and then retreats, indicating the presence of a rather sharp encircling ridge under the cuticle immediately behind the constriction. Behind the ridge is an almost imperceptible, very shallow, second constriction. Notwithstanding these changes in the countour of the interior portion of the nema, the contour of the body itself varies but very little, there being only a decrease in diameter, sometimes almost imperceptible, opposite the before-mentioned ridge. Another marked difference in these larvae is the hooked condition of the tail end, which has somewhat the form of a fishhook, the curvature being all of 180°. The terminus is acute. Almost at the terminus on the ventral side there is a slight irregularity in the ventral contour, so that there is a slight asymmetry at the apex.

It should be mentioned that the number of elements (cells?) which make up the node is subject to some variation in both types of larvae, and that sometimes larvae of the larger type have the hooked caudal extremity nearly as pronounced as those of the smaller type. Except for size, the two types of larvae are certainly very similar morphologically, a condition which is equally marked in the adults.

[5] The term "trophocytes", as used by Cobb in this connection, refers to the cells or elements in the postnodal part of the body and not to structures associated with the modified intestine or trophosome.

On January 4, 1928, four nymphs of *Melanoplus femur-rubrum* were exposed to active preparasitic larvae of the smaller type. An attempt was made to infest the nymphs with one parasite each. The grasshoppers were dissected on February 16. Two were not parasitized, one harbored a female mermithid 16.5 cm long, and one a female mermithid 35 cm long. This experiment should, of course, be repeated and the mermithids reared to maturity. However, several hundred experimental females have been kept under observation by the writer, and eggs of the smaller type have never been deposited by females exceeding 15 cm in length. The rearing of a female 35 cm long from a preparasitic larva of the smaller type does not support the contention that these smaller larvae are a different species. The writer is inclined to regard the size differences of eggs and preparasitic larvae as due to some host influence. It is one of the many unsolved problems that await future investigators of this group of parasites.

PARASITIC LARVAL STAGE

The rate of parasitic larval development is subject to considerable variation. This is due in part to individual differences in the larvae, for parasites that enter the same host at the same time do not develop at the same rate. The age and physical condition of the host are also important factors. When newly-hatched grasshopper nymphs are infested the rate of development of the parasites is somewhat slower than when the nymphs are several days old. If infested nymphs are subjected to a temperature low enough to render them inactive and reduce food consumption the development of the parasites is noticeably retarded.

THREE DAYS IN THE HOST

There is little change in the length of the larva during the first 3 days in the host. The width increases slightly. The subdorsal esophageal gland is less conspicuous, although remnants of it can usually be seen. In one specimen, examined alive, the outline of the entire gland was visible. Although appearing nearly empty it still contained scattered masses of what seemed to be partly degenerated globules which moved backward and forward with the movement of the animal. Traces of the subventral glands may sometimes be seen, but in most cases they have disappeared. Conspicuous unicellular structures occur in the esophageal region of many mermithids and have been termed "stichocytes" by Steiner (*20*). They may occur as isolated structures or, as in the present case, they may lie close together and form a distinct body which has been called a "stichosome." Recent investigations by Chitwood (*1*) have demonstrated that each stichocyte is an esophageal gland and communicates with the lumen of the esophagus through a pore. After *Agamermis decaudata* has been 3 days in the host the stichosome contains 16 large and conspicuous nuclei, but the contour of the individual stichocytes is obscure.

FOUR DAYS IN THE HOST

The length of a 4-day parasite (fig. 8) is still not appreciably greater than that of a preparasitic larva from the anterior end to the node. Its maximum diameter has increased and is now 25μ to 40μ. The esophageal bulb has increased somewhat in length and its anterior part is drawn out into a long neck. It still undergoes rhythmic mus-

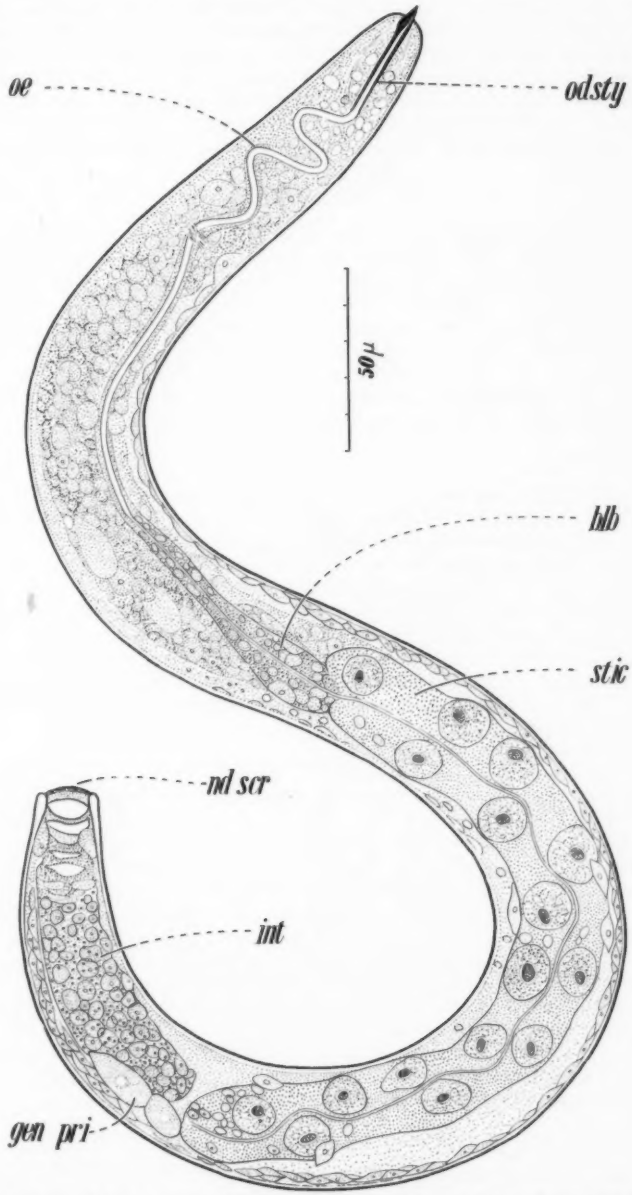

FIGURE 8.—Parasitic larva of *Agamermis decaudata* 4 days in the host: *bib*, Bulblike enlargement of esophagus; *gen pri*, genital primordium; *int*, intestine; *nd scr*, nodal scar; *odsty*, odontostyl; *oe*, esophagus *stic*, stichocytes (esophageal glands).

cular contractions. The granules or globules characteristic of the preparasitic larva have largely disappeared. No trace of the subdorsal and subventral esophageal glands was seen. The nuclei of the stichocytes are spherical or slightly oblong and have a diameter of about 12μ. Throughout the length of the esophagus the lumen is distinct. The intestine is composed of distinct, nucleated cells. The size and appearance of the genital primordium remain unchanged.

SIX DAYS IN THE HOST

After 6 days the larvae studied (fig. 9) were 660μ to 680μ long by 50μ to 60μ wide. The intestine is a distinctly cellular organ without a lumen. The stichosome, with its 16 nuclei, is now a large and conspicuous body. Although the lumen of the esophagus is distinctly seen, the esophageal tissues surrounding it are obscure and are more or less embedded in the stichosome. This fact is not apparent in preparations in toto, where the esophageal tube appears to pass directly through the stichosome. One is reminded of a similar condition in members of the genus *Trichuris*.

EIGHT DAYS IN THE HOST

After 8 days the specimens studied (fig. 10) averaged 1.5 mm in length by 70μ in width. The region of the body from the anterior end to the esophageal bulb is slightly shorter than the corresponding region in a preparasitic larva. The region containing the posterior portion of the esophagus is about 100μ longer than that region in the preparasitic larva. The greatest increase in length was in that part of the body containing the intestine. This is the beginning of an enormous growth in the intestine. The boundaries of the individual stichocytes are faintly visible. The genital primordium is slightly larger and it is now situated somewhat posterior to the junction of esophagus and intestine.

FOURTEEN DAYS IN THE HOST

After 14 days the specimens studied (fig. 11) were 3.5 to 4 mm long by 80μ to 90μ wide. The anterior portion of the esophagus is 350μ long, with an average diameter of 20μ; its course is tortuous, and the esophageal tube is distinct. The posterior portion of the esophagus is more or less cylindrical, 750μ long by about 10μ to 12μ wide, with a small but distinct lumen. The boundaries of the individual stichocytes are fairly distinct. The body posterior to the base of the esophagus is filled with a greatly enlarged intestine in the form of a cylindrical mass of cells and is without a lumen. Its cells have pushed past the base of the esophagus and extend anteriad nearly to the nerve ring. The genital primordium has shifted posteriad with the growth of the intestinal tissue and now lies about midway between the base of the esophagus and the posterior end of the body. It is still an oblong structure, usually about 90μ long by 30μ wide.

DURATION OF PARASITIC LARVAL STAGE

Infestation may take place in nature over a considerable period. In one instance a female larva emerged from a nymph of *Melanoplus femoratus* Burm. on July 15. Assuming that this parasite required 80 days to complete its development in the host, infestation must

have taken place during the latter part of April. Parasites that have not been in the host more than 3 or 4 days are occasionally dissected from grasshoppers as late as August. Nevertheless, in northeastern

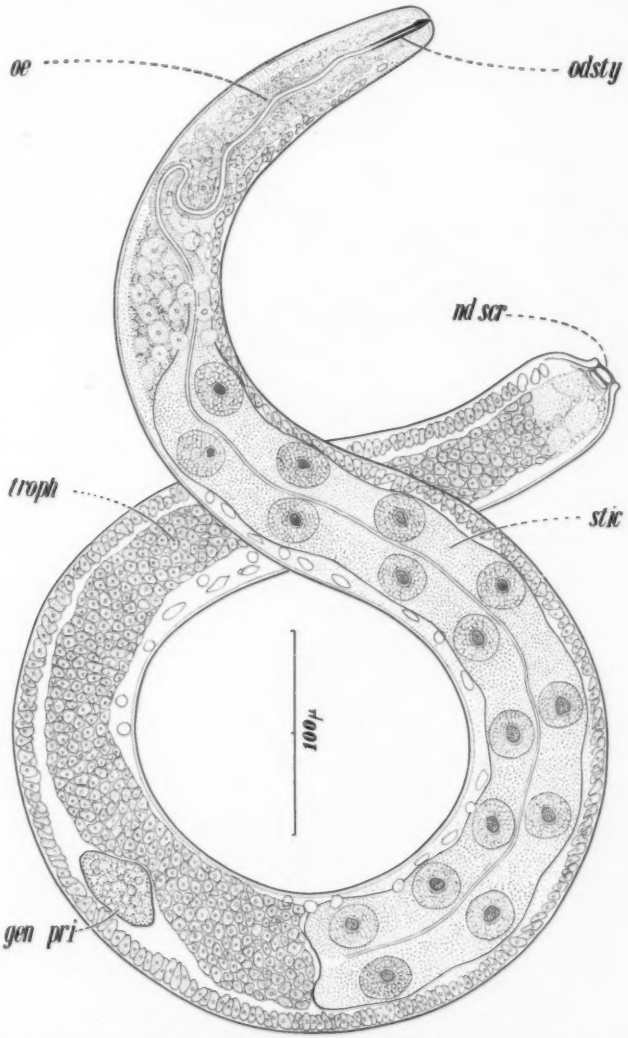

FIGURE 9.—Parasitic larva of *Agamermis decaudata* 6 days in the host: *gen pri*, Genital primordium; *nd scr*, nodal scar; *odsty*, odontostyl; *oe*, esophagus; *stic*, stichosome composed of 16 stichocytes (esophageal glands); *troph*, trophosome (modified intestine).

Virginia a large percentage of the infestation, so far as grasshoppers are concerned, probably takes place during about 2 weeks near the middle of June. All the parasitic larvae dissected at a given time of

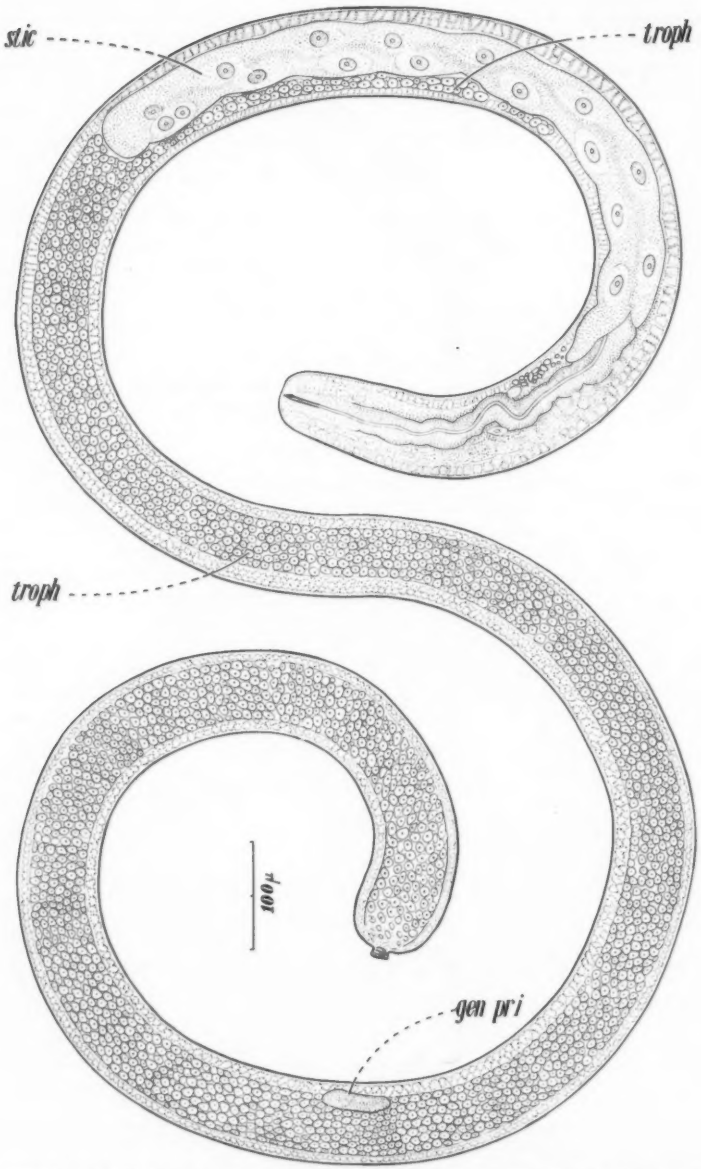

FIGURE 10.—Parasitic larva of *Agamermis decaudata* 8 days in the host: *gen pri*, Genital primordium; *stic*, stichosome; *troph*, trophosome.

the year from grasshoppers collected in a particular locality show a tendency to be in approximately the same stage of development. This is in marked contrast to the case of *Mermis subnigrescens*, where it is common for a host to harbor parasites in widely different stages of development.

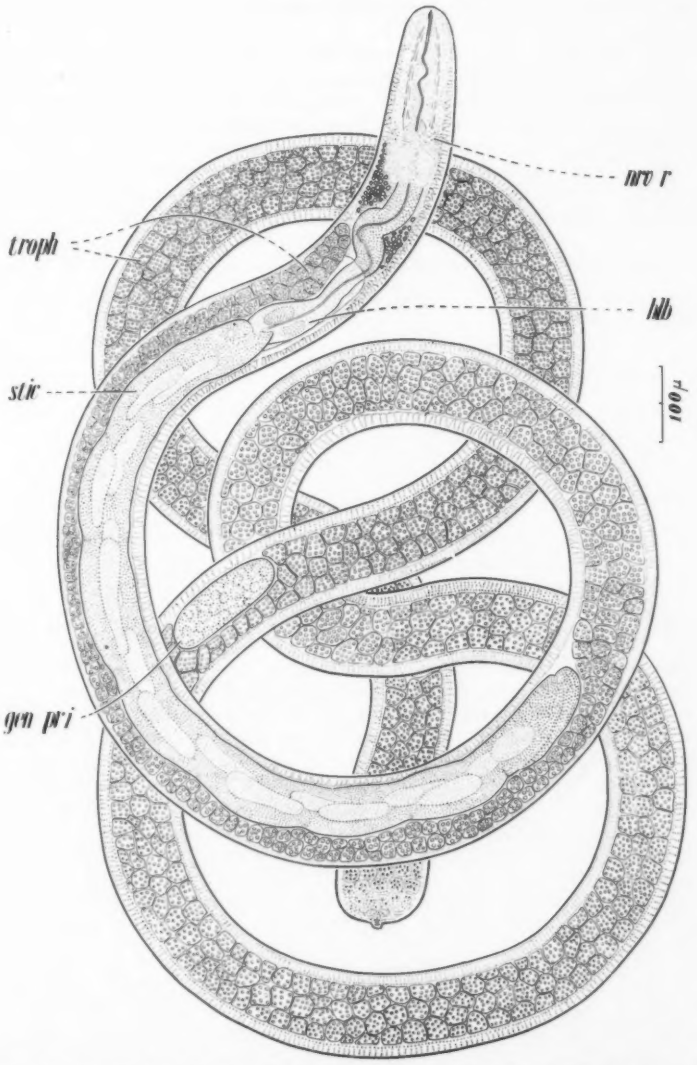

FIGURE 11.—Parasitic larva of *Agamermis decaudata* 14 days in the host: *blb*, Bulblike enlargement of esophagus; *gen pri*, genital primordium; *nrv r*, nerve ring; *stic*, stichosome (esophageal glands); *troph*, trophosome.

When grasshoppers were collected in early August and confined in cages, the parasites that emerged during the following week included many males. When the grasshoppers were collected during the latter part of August, the parasites were largely females, and when the grasshoppers were collected during September, the parasites were almost exclusively females. Table 2 is based on nymphs of *Melanoplus femur-rubrum* that were reared in the laboratory and experimentally infested.

In cases where both males and females are harbored by the same host, all frequently emerge simultaneously at about the normal time for the emergence of the males. Some of these prematurely emerged females survive and some die, depending, apparently, on the extent of development at the time of emergence.

While the duration of the parasitic stage is subject to considerable variation, it is usually from 1 to 1½ months for males and from 2 to 3 months for females.

TABLE 2.—*Duration of the parasitic larval stage of Agamermis decaudata*

Parasites per host				Period from infestation to emergence	Parasites per host				Period from infestation to emergence
Females	Males	Sex not determined	Total		Females	Males	Sex not determined	Total	
Number	*Number*	*Number*	*Number*	*Days*	*Number*	*Number*	*Number*	*Number*	*Days*
----------	2	2	4	30	1	----------	----------	1	81
----------	2	----------	2	33	1	----------	----------	1	81
2	2	----------	4	38	----------	2	----------	2	35
----------	2	----------	2	44	----------	5	1	6	31
----------	2	----------	2	31					

EFFECTS OF THE PARASITE ON THE HOST

There is no doubt that grasshopper nymphs can be infested with a sufficient number of *Agamermis decaudata* to cause the death of the host. It is difficult to determine how many are necessary. The writer at one time regarded from 8 to 10 as usually constituting a lethal infestation, but this estimate was based on hosts reared in the laboratory and may not be true for grasshoppers in their natural environment. The number of parasites that an animal will sometimes tolerate is one of the astonishing things about parasitism.

Although over 3,000 infested grasshoppers, collected in the vicinity of Falls Church, Va., have been examined, only 1 harbored as many as 4 *Agamermis decaudata* parasites, and instances of 2 and 3 parasites per host were comparatively rare. But, strangely enough, on one occasion 7 larvae of this species were dissected from the small coccinellid beetle *Ceratomegilla fuscilabris* Mulsant. That grasshoppers can be infested with a large number of parasites of this species has been demonstrated in the laboratory, where as many as 27 larvae have entered 1 nymph. However, one parasite per host (fig. 12) appears to be the usual number in nature, and evidently death from overinfestation is not a common occurrence.

Mermithids usually kill their host at the time they emerge. Crawley and Baylis (*6*), in the case of ants, found that "after the emergence of the worm the ants became very active but their death was only

deferred a short time." Strickland (*21*) states that *Simulium* larvae were always finally killed when the mermithid bored its way out to escape into the water. Glaser and Wilcox (*8, p. 13*) observed, in the case of *Melanoplus atlanis* and *M. bivittatus*, that "when the worms are about to emerge, the grasshoppers fall over on one side, kick for a time, and then die."

The writer has observed the emergence of *Agamermis decaudata* many times. The parasites came out head foremost, forcing their way through the body wall between the segments, most often in the region of the thorax. In no case were they seen emerging through the mouth or anus. A half hour or more was often required for the process, during which time the grasshopper might be quite active, dragging the mermithid around and otherwise treating it roughly, a fact which may account for the rather common occurrence in nature of injured postparasitic larvae and adults. In some cases the nema, when about half way out, coiled around a blade of grass and entirely extricated itself as the grasshopper moved away.

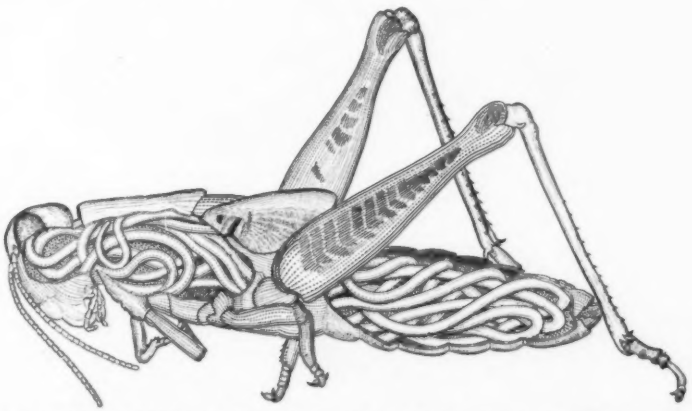

FIGURE 12.—Nymph of *Melanoplus femur-rubrum* with parts of body wall dissected away to show position of parasite. This grasshopper contains one female *Agamermis decaudata*, which is approximately full-grown.

The immediate effect upon the grasshopper is a partial paralysis of the legs and an inability of the insect to maintain its equilibrium. It will fall over on one side, then right itself and crawl slowly around, using its fore and middle legs with apparent difficulty and often dragging its hind legs. These movements become more sluggish until finally it falls over and remains quiet as if dead, but will move if disturbed, and may live for several hours. A grasshopper from which a mermithid has emerged during the day may still be alive when the laboratory is closed for the night, but it is always dead the following morning.

The external structure of some insects is noticeably changed by the presence of mermithid parasites. This is especially true in the case of ants. Inasmuch as Wheeler (*22*) has carefully discussed this phase of the subject, it will not be reviewed here. The writer has not been able to detect, at any stage in their development, a noticeable external difference in structure between parasitized and nonparasitized grasshoppers.

In every case where the writer has examined an adult female *Melanoplus femur-rubrum* parasitized with *Agamermis decaudata*, the ovaries were reduced in size (fig. 13), and in many instances were vestigial. The extent to which the female gonads are reduced depends on the time the infestation was acquired and the number and size of the parasites. In the case of *Mermis subnigrescens*, where infestation may take place at any stage in the life of the host, the time of infestation and the number of parasites become important factors in this connection. With *A. decaudata*, of which one parasite each is usually acquired while the nymphs are young, these factors cause less variation in the female gonad development of their hosts. When an adult grasshopper is found parasitized with a single *A.*

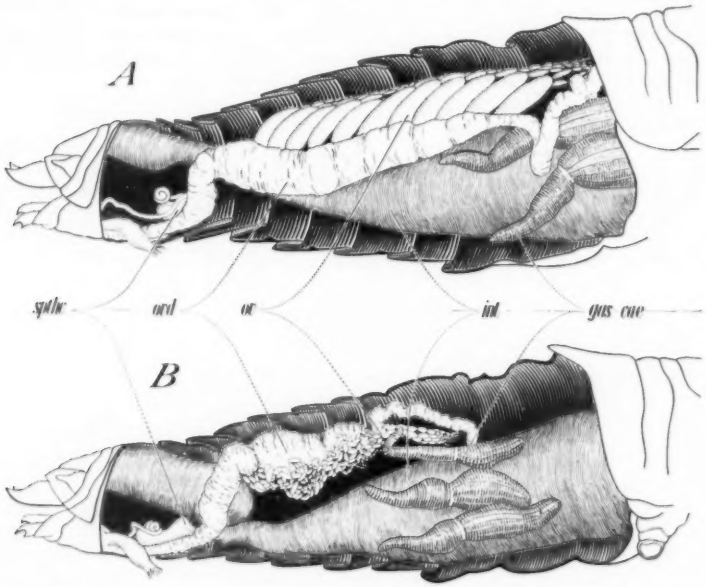

FIGURE 13.—*Melanoplus femur-rubrum* dissected to show female reproductive organs of (*A*) normal adult and (*B*) one from which *Agamermis decaudata* has emerged. *Gas cae*, gastric caeca; *int*, intestine; *ov*, oval; *ovd*, oviduct; *spthc*, spermatheca.

decaudata the parasite is usually a female. This is probably because males of this species, having a shorter parasitic stage, usually emerge before their hosts reach maturity. Consequently the cases of female gonad suppression that have come to the writer's attention have been those in which grasshoppers harbored relatively large mermithids. To what extent the smaller male parasites affect the female gonad development of their hosts is not known.

The male gonads of *Melanoplus femur-rubrum* are much less affected by the presence of mermithids than are the female gonads. In many instances the testes are not materially reduced in size, and all parasitized male grasshoppers examined by the writer seemed capable of producing spermatozoa. In several instances copulating pairs of both

M. femur-rubrum and *M. bivittatus* have been captured, and on examination the male was found to harbor a mermithid.

Specimens of *Melanoplus femur-rubrum* collected from the middle to the latter part of September were largely adults, with a few individuals still in the nymphal stage. In several instances nearly all such nymphs were parasitized, although not over 10 percent of the adults were infested. The probable explanation of this became apparent when it was noted that in cage-reared grasshoppers parasitized individuals were still in the nymph stage long after uninfested individuals, hatched at the same time, had reached maturity. This retarded development of infested grasshoppers is sometimes very pronounced.

EFFECTS OF THE HOST ON THE PARASITE

In an earlier paper (*2*) it was demonstrated that in the case of *Mermis subnigrescens* a large number of mermithids per host resulted in the development of male parasites and a small number of mermithids per host in the development of females. It was suggested that this is probably also true in the case of *Agamermis decaudata*. The manner in which *M. subnigrescens* is acquired often results in successive infestations, and a single host may harbor 20, 30, or, on rare occasions, over 100 parasites. In the case of *A. decaudata*, as already noted, a grasshopper harboring more than two or three parasites has rarely been found. Nevertheless, males of *M. subnigrescens*, while easily reared in the laboratory, are seldom encountered in nature, few specimens ever having been collected. Males of *A. decaudata*, on the other hand, are always more numerous than the females and are easily collected in large numbers. Males of this species can and do develop in grasshopper hosts, although when these insects are collected and confined in cages the number of males that emerge is surprisingly small. But insects other than grasshoppers are infested with *A. decaudata*. In the vicinity of Falls Church, Va., the coccinellid beetle *Ceratomegilla fuscilabris* and the leaf hopper *Draeculacephala mollipes* (Say) both regularly harbor this parasite, and on several occasions unidentified crickets have been found infested. When the coccinellid beetles were collected and confined in cages, the few parasites that emerged were males. It seems probable, therefore, that some of these smaller insect hosts contribute toward the development of males of *A. decaudata*.

COMPARATIVE INFESTATION OF MALE AND FEMALE GRASSHOPPERS

When examining *Melanoplus bivittatus* and *M. atlanis* collected near Dummerston Station, Vt., Glaser and Wilcox (*8*) found parasitized, on an average, about 45 percent of the females but only about 9 percent of the males. The data in table 3, based on an examination of over 3,000 specimens composed largely, but not exclusively, of three species, namely, *M. femur-rubrum*, *Orchelimum vulgare*, and *Conocephalus brevipenne* (Scudder), show no such difference between the percentage of infestation in the two sexes.

Table 3 was compiled from dissections begun in early July and continued throughout the year as long as grasshoppers could be collected. A large percentage of the material examined, however, consisted of nymphs.

POSTPARASITIC LARVAL STAGE

MIGRATION TO THE SOIL

Once free from the host, the postparasitic larvae enter the soil. This migration is probably one of the vulnerable points in the life cycle. In breeding cages where the soil is hard or crusted over, many newly emerged parasites perish, and one often finds their dried and tightly coiled remains on the surface of the soil or clinging to foliage put in to serve as food for the grasshoppers. It seemed possible that wet or humid conditions might stimulate fully grown parasitic larvae to emerge, and the writer attempted to test this by thoroughly wetting cages containing infested grasshoppers, but was unable to show in a convincing manner that the water had any effect. In every case where emergence has been observed it took place during the day, showing that this act is at least not confined to the night. In nature, the soil ordinarily is not very hard or dry beneath thick herbaceous vegetation and the ground is penetrated to a surprising extent by the galleries of various organisms.

TABLE 3.—*Comparison of the number of male and female grasshoppers infested with Agamermis decaudata*

Family	Males			Females		
	Total examined	Infested		Total examined	Infested	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Acrididae [1]	981	105	10.7	1,304	134	10.2
Tettigoniidae [2]	367	72	19.6	457	67	14.6

[1] Largely *Melanoplus femur-rubrum*.
[2] Largely *Orchelimum vulgare* and *Conocephalus brevipenne*.

Females usually penetrate the soil to a depth of from 5 to 15 cm, where they coil themselves into the characteristic knot (fig. 14). Here they molt, copulate, deposit their eggs, and die. The vertical distribution of these mermithids beneath an area of 9 square feet in a field at Falls Church, Va., is shown in table 4. Both adults and postparasitic larvae are included.

TABLE 4.—*Vertical distribution of Agamermis decaudata in the soil*

Depth (cm)	Males	Females	Total	Depth (cm)	Males	Females	Total
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
1 to 5	1	2	3	20 to 25	1	1	2
5 to 10	37	24	61	25 to 30	0	0	0
10 to 15	12	6	18				
15 to 20	4	1	5	Total	55	34	89

Males apparently do not seek the females until the latter have molted or are about to molt, and during the first winter individuals of the two sexes remain isolated. The knot of the postparasitic larval female is composed of loose loops, in contrast to the tight knot usually formed by adults.

DURATION OF THE POSTPARASITIC LARVAL STAGE

Like other stages in the development of this parasite, the duration of the postparasitic larval stage varies somewhat with different indi-

FIGURE 14.—A knot of *Agamermis decaudata*, containing a female and several males, in natural position in the soil.

viduals. A majority of the females molt during July and begin depositing eggs in August. Information regarding males is less satisfac-

tory, but their postparasitic larval development appears to parallel that of the females (table 5).

TABLE 5.—*Duration of the postparasitic larval stage of Agamermis decaudata*

Larva no.	First examination June 8, about 9 months after emergence	Second examination July 11, about 10 months after emergence	Third examination August 6, about 11 months after emergence
1	Molt starting, specimen injured and preserved.		
2	No sign of an approaching molt.	Outer cuticle separating at anterior and posterior ends.	Cuticle not entirely off.
3	Molt fairly well under way. Nema in poor condition. Preserved.		
4	Cuticle separating at extremities of body.	Lost.	
5	No sign of approaching molt	Outer cuticle separated at anterior end.	Completely molted. Eggs not seen.
6	do	Not examined	Completely molted. Had already deposited eggs. Oldest embryos 6 to 8 days old.
7	do	Completely molted	Lost.
8	do	Outer cuticle separated at extremities of body.	Completely molted. Had not deposited eggs; probably nearly ready to do so.
9	do	Not examined	Completely molted, deposited numerous eggs. Oldest embryos 8 to 10 days old.
10	do	Molt complete	Lost.

The duration of the postparasitic larval stage of females is about 9 to 11 months and, roughly, extends from September to July. This appears to be true for males also, although the time of their molting has not been carefully determined.

THE NODAL SCAR

The elements that constitute the node of the preparasitic larva form a plug to prevent the body contents from exuding after the caudal portion of the body is broken off. This structure persists as the nodal scar throughout larval development. It is a conspicuous anatomical feature while the larva is small but becomes increasingly less conspicuous as the larva grows, and is difficult to see in the postparasitic larval stage. Its presence constitutes convincing evidence that no molt takes place during parasitic development.

Immediately before the final molt, females have a yellowish or brownish tinge. The old cuticle first separates at the extremities of the body, then breaks transversely a short distance posterior to the head, and the anterior and posterior portions come off over the head and tail respectively. In males, the yellowish tinge preceding the final molt is not so noticeable but the cuticle is shed in a similar manner and at approximately the same time as is that of the females.

ADULT STAGE

At the time of the final molt both males and females are very opaque. This opaqueness is due primarily to the trophosome or fat body (modified intestine), which fills all available space in the body. This structure probably contains the reserve material for all future metabolism. As this reserve material is gradually consumed the body of the mermithid becomes less and less opaque, and when it is exhausted the animal dies.

In the case of these recently molted first-year females, oviposition begins nearly a year after emergence from the host, sometimes in July but usually during August, and continues until interrupted by cold weather. It is resumed the following June.

Three gravid females were dug from the soil on November 27, and washed free of eggs. Each female, together with the accompanying males, was placed in a cavity between two disks of soil, and each pair of soil disks was placed in a tin box. The boxes were buried 15 cm below the surface of the soil. From February until July these specimens were examined at intervals of about 2 weeks. Eggs were first seen on July 12, although it was believed that in all three cases egg laying had begun 10 to 12 days earlier.

The foregoing experiment, corroborated by field observations, indicates that second-year females begin depositing eggs somewhat earlier in the summer than do recently molted first-year females. At the end of the second summer in the soil, females are very transparent and the trophosome is nearly exhausted. Experimental animals usually failed to survive a third winter; two that were recovered alive the following spring were in a nearly exhausted condition and seemed incapable of depositing more eggs.

A female collected during the winter is surrounded by all the eggs deposited during the preceding summer. Egg counts were made on six females by the following method. Each knot, together with the surrounding soil, was placed in a graduated cylinder, and water was added to make 100 cc. After a thorough shaking, a 5-cc sample was removed; the eggs were counted and the total number computed. The totals were 2,625, 6,530, 5,420, 5,680, 4,780, and 3,430 respectively. As eggs are deposited during two summers, these values may represent about half the total output.

In a former paper (*2*) it was pointed out that when females are not permitted to copulate they do not deposit eggs. Virgin females, after remaining in the soil for as long as 14 months, oviposit when males are provided. In view of the prevalence of males, it seems unlikely that in nature females are often rendered unproductive through lack of mates. Each female is usually accompanied by from 2 to 4 males, and in one instance a knot was found composed of a female and 24 males. Occasionally 2 females will be found in the same knot, but such instances are rare.

As previously noted, second-year females do not usually resume egg laying until June. Females kept in the laboratory at room temperature deposit eggs at any time throughout the year. The suppression of egg laying during the winter seems, at first glance, to be a matter of temperature. However, moisture is probably also an important factor. If the soil in which ovipositing females are kept is moistened beyond a certain point egg laying stops. In experimental work considerable care must be exercised in regulating moisture if normal egg laying is to be maintained. In the vicinity of Falls Church, Va., *Agamermis decaudata* is confined to the so-called "bottom" lands. The soil in these localities is usually saturated with water throughout the winter and is frequently flooded for short periods. By June the soil usually has become sufficiently dry to be tillable. Under these conditions it seems probable that moisture is an important factor in retarding egg laying during early summer.

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SUMMARY OF LIFE CYCLE

The following summary of the life cycle of *Agamermis decaudata* is based on observations made at Falls Church, Va. The grasshoppers referred to are *Conocephalus brevipenne* and *Melanoplus femur-rubrum*, two of the commonly infested species in this locality. The different changes are indicated as taking place during certain periods at various times of the year. Although the outline as given is essentially correct, occasionally an individual may undergo a certain change before or after the time indicated. A few preparasitic larvae enter hosts at least as early as April or as late as August, and the time of emergence is correspondingly affected. All males do not necessarily emerge during July nor all females during August. It is not known to what extent the life cycle may be changed when insects other than grasshoppers serve as hosts.

August.—Knot *a* (fig. 15) is composed of one female *Agamermis decaudata* and several males that emerged from their hosts 2 years previously. The female began depositing eggs during August of the preceding year. Knot *b* is composed of one female and several males that emerged from their hosts 1 year previously and molted the preceding July. The female is now beginning to deposit eggs for the first time. Parasites of the present season, which would now be emerging, are omitted for the sake of brevity. After these have emerged and before those in knot *a* have died there exist simultaneously in the soil mermithids of three ages. Grasshoppers are also depositing eggs, *Melanoplus femur-rubrum* in the soil and *Conocephalus brevipenne* in the stems of plants.

September and October.—The female mermithid in knot *a* and the one in knot *b* (fig. 15) continue to deposit eggs, and by October several thousands have accumulated around each.

November and December.—The females of knots *a* and *b* (fig. 16) stop laying eggs with the advent of cold weather. The mermithids in knot *a* have nearly exhausted their supply of stored food material and will not survive the winter.

January and February.—An occasional egg from knots *a* and *b* (fig. 16) may hatch.

March and April.—A few eggs from knots *a* and *b* (fig. 16) continue to hatch, the number increasing slightly.

May.—Grasshopper eggs begin to hatch during the latter part of the month. There is an increase in the number of *Agamermis decaudata* eggs which are hatching, and the infestation of nymphs begins.

June.—The remaining eggs in knots *a* and *b* (fig. 17), which comprise a very large percentage of those present the preceding autumn, now hatch. The larvae migrate to the surface of the soil, then seek out and enter the young grasshopper nymphs, probably climbing the vegetation while it is wet with dew or rain.

Eggs are hatching which have been deposited by females of two ages, namely, the female *a*, which emerged about 31 months previous and is now dead, and the female *b*, which emerged about 19 months previous and which will continue to deposit eggs throughout the summer.

July.—The eggs in knots *a* and *b* (fig. 17) have all hatched and the period of infestation is over except for an occasional belated infestation. The female in knot *b* now resumes egg laying, not having deposited eggs since the previous November.

August.—Uninfested grasshoppers are now depositing eggs. Larval male mermithids are emerging from their hosts and entering the soil.

September.—Larval female mermithids are emerging from their hosts and entering the soil.

October.—The female in knot *b* (fig. 18) continues to deposit eggs. Newly emerged males (*c*, ♂) and females (*c*, ♀) form separate knots in the soil.

November and December.—The female in knot *b* (fig. 19) stops laying eggs; she is now surrounded by several thousand eggs.

January and February.—An occasional egg from knot *b* (fig. 19) may hatch.

March and April.—Eggs from knot *b* (fig. 19) continue to hatch, the number increasing slightly.

May.—Grasshoppers now begin to hatch. The eggs from knot *b* (fig. 20) hatch in increasing numbers and the infestation of nymphs begins. The mermithids in knot *b* are dead.

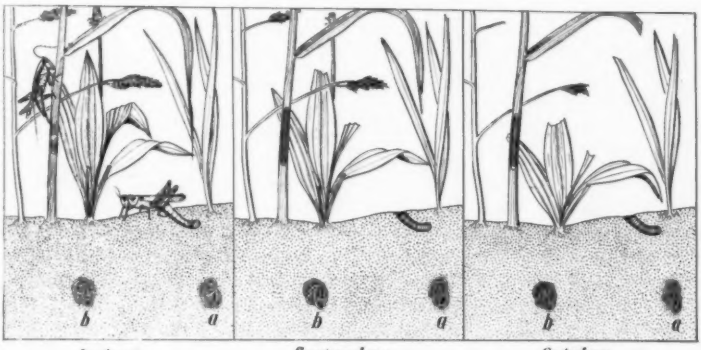

FIGURE 15.—Life cycle of *Agamermis decaudata*. August to October. *a*, Knot composed of one female and several males that emerged from their hosts 2 years previously; *b*, knot composed of one female and several males that emerged from their hosts 1 year previously.

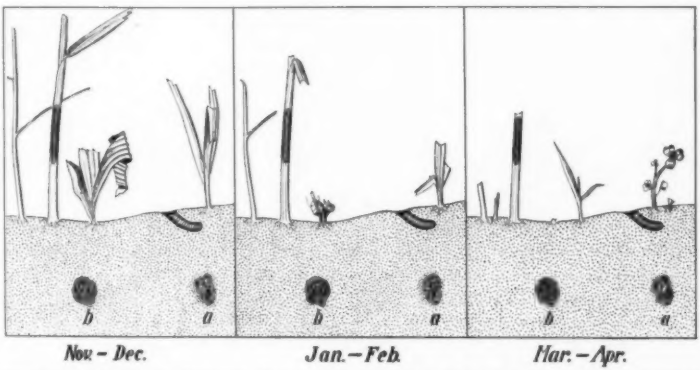

FIGURE 16.—Life cycle of *Agamermis decaudata*, November to April. *a* and *b*, Knots as described in figure 15.

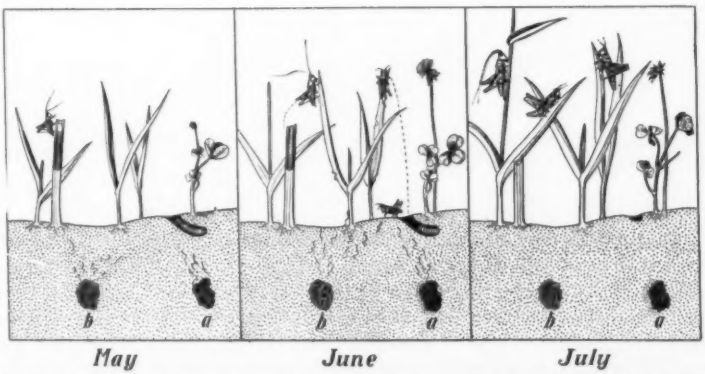

FIGURE 17.—Life cycle of *Agamermis decaudata*, May to July. Larvae issuing from knots at *a* and *b*; female in knot *a* now dead; female in knot *b* resumes egg laying.

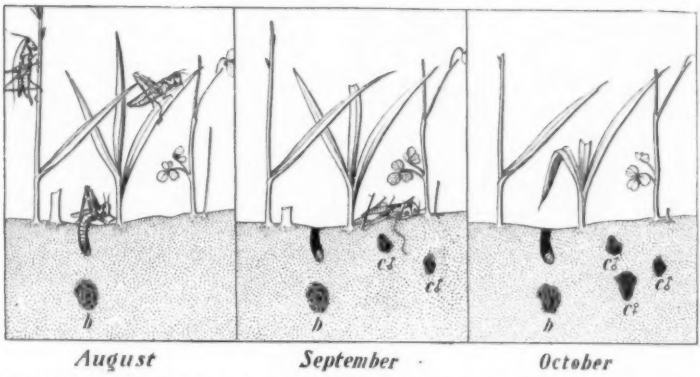

FIGURE 18.—Life cycle of *Agamermis decaudata*, August to October. Female in knot *b*, continues to deposit eggs; males (*c*, ♂) and females (*c*, ♀) emerge from grasshoppers and form separate knots.

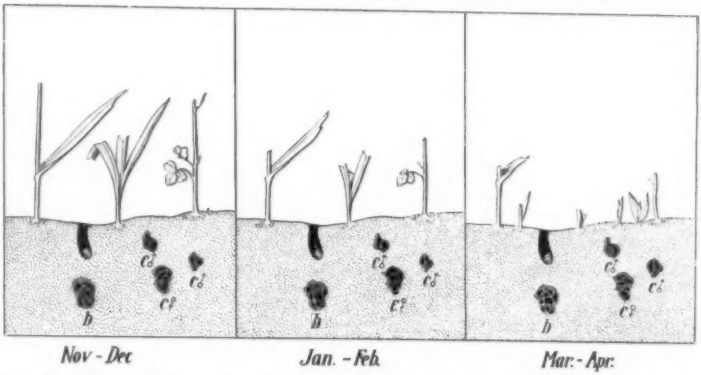

FIGURE 19.—Life cycle of *Agamermis decaudata*, November to April. *b*, Knot containing female and several thousand eggs; *c*, ♂, and *c*, ♀, knots containing male and female larvae, respectively.

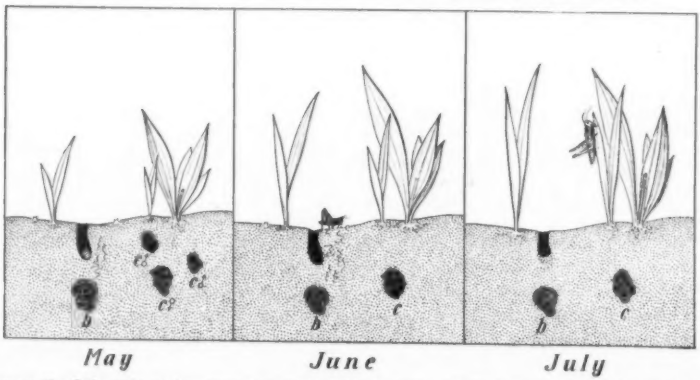

FIGURE 20.—Life cycle of *Agamermis decaudata*, May to July. Adults in knot *b* dead; larvae issuing from knot *b*; males from knot *c*, ♂, join females in knot *c*, ♀, forming knot *c*.

June.—The remaining eggs from knot *b* (fig. 20) now hatch, and the infestation of grasshopper nymphs takes place as during the previous June. The more recently emerged postparasitic larvae (*c*, ♂, and *c*, ♀) are preparing to molt and males have joined or are about to join the females.

July.—The female and males in knot *c* (fig. 20) are now molting and the female will begin to deposit eggs during August.

SUMMARY

Mermithids are common nematode parasites of many insects. *Agamermis decaudata*, a parasite of grasshoppers and some other insects, is found throughout the northeastern part of the United States, southward to Virginia and westward to Nebraska. The present paper is based on studies made at Falls Church, Va.

The adults of this parasite occur in the soil, where eggs are deposited. Hatching takes place between late June and mid-July. Larvae migrate to the surface of the soil, climb the vegetation, and enter the body cavity of recently hatched grasshopper nymphs by penetrating the body wall. The parasites remain in the host from 1 to 3 months and complete their growth. They then emerge by forcing their way through the body wall and enter the soil. Molting takes place the following summer and females begin depositing eggs.

Infested grasshoppers are retarded in their development and females are usually rendered sterile. The emergence of the parasite causes the death of the grasshopper. The morphological development of the parasite is discussed.

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CHROMOSOME DIFFERENCES IN A WHEAT-RYE AMPHIDIPLOID [1]

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INTRODUCTION

Plant hybrids that contain the diploid chromosome complement of the parents have been described in recent years by a number of investigators. They occur rarely and are produced in combinations which usually are highly infertile or self-sterile in the F_1 generation. Some investigators have designated such hybrids as "amphidiploids" to call attention to the presence in one plant of both parental chromosome sets in the diploid condition. Others, principally the Russian workers, have called them "balanced hybrids" to emphasize the paired or balanced condition of the chromosome elements.

Most of the known amphidiploids have been produced from interspecific and intergeneric artificial hybrids. They are of special interest to geneticists and cytologists on account of the light they shed on methods of species formation and also on account of their possibilities for plant improvement.

LITERATURE REVIEW

Meister (*3*)[3] first reported in 1927 the discovery by his associates and himself of a 56-chromosome wheat-rye amphidiploid.

Tjumjakoff (*9*) mentioned the probable polyploid nature of the hybrids, and Meister (*4*, *fig. 6*) showed a spike of this unusual type of wheat-rye hybrid, stating that it was apparently of tetraploid make-up.

Meister (*5*) gave a botanical description of the Saratov wheat-rye amphidiploids and designated the new species combination as *Triticum secalotricum saratoviense* Meister.

The polyploid nature of these constant, fertile or partially fertile hybrids was determined by the cytological investigations of Levitsky and Benetzkaia (*2*). They found in the somatic cells of the hybrid 56 chromosomes and in the gametic cells 28 chromosomes. The somatic number given was established for nine individuals of the F_4, F_5, and F_6 generations in three different families.

In this material they observed both normal and irregular chromosome behavior in the meiotic divisions. In some division figures from two to six or more lagging univalents were found. The univalents afterward split longitudinally, sometimes with irregular scattering of

[1] Received for publication Oct. 3, 1935; issued March 1936.

[2] The writer wishes to express appreciation to C. W. Hungerford, chief of the Division of Plant Pathology and dean of the Graduate School, University of Idaho, for the use of his excellent research microscope and for laboratory space and equipment; also to J. W. Taylor, associate agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for supplying the hybrid material which made this work possible.

[3] Reference is made by number (italic) to Literature Cited, p. 204.

chromosomes over the spindle. Chromosome elimination into the plasma was observed, also complete disintegration of amorphous elements, such as microcytes, cell fragments, etc.

Judging by the number of lagging chromosomes, Levitsky and Benetzkaia estimated that probably at least 50 percent of the pollen grains contained an increased number of chromosomes (more than 28), yet all of the parental plants examined had 56. They conclude:

> Thus it remains to suppose that these normal gametes, having arisen from normal divisions, leave behind with their pollen tubes all other gametes with deviating chromosome numbers * * *.

The formation of amphidiploids is believed by these authors to result from "apogamous development of the ovule in F_1."

Tjumjakoff (*10*), in a study of the fertility of these wheat-rye hybrids of balanced type, found in each of two lines that the average number of kernels per spikelet in normal spikes was 1.2. The following year the average number of kernels per spikelet of the F_4, when sown in pots, was 0.9. In the former this would be a fertility of about 50 to 60 percent for the common wheats under average conditions.

A brief description of the plant characters and of certain chromosome differences in an artificially produced wheat-rye amphidiploid is reported herein.

MATERIAL AND EXPERIMENTAL DATA

An individual plant head of a wheat-rye hybrid having the hairy-neck rye character was obtained from the Arlington Experiment Farm, near Washington, D. C., in the winter of 1930. This hybrid had been under observation for a number of years by J. W. Taylor, who made the original wheat-rye cross, the backcrosses, and the selection.

The origin of this amphidiploid was indicated by Taylor and Quisenberry (*8*) in a report on the inheritance of rye crossability in wheat hybrids. Purplestraw wheat was crossed with Abruzzes rye in 1923. The F_1 was backcrossed to Purplestraw, producing two seeds from more than 1,000 pollinations. The progeny of one of these seeds, consisting of 10 plants, was backcrossed with rye Only two of the 20 F_1 hybrid plants from backcrossing with rye dehisced and one of these produced a single selfed seed. Taylor and Quisenberry state: "From this seed originated a so-called nonsegregating line intermediate between wheat and rye" (*8, p. 150*).

Plants from this amphidiploid were grown in the agronomy garden of the University of Idaho for 2 years. In both years they were low in vigor at the beginning of the growing season, but before the end of the season they developed into fairly vigorous plants, which in most characters were intermediate between the parents.

The leaves and culms were faintly to definitely purple in the early stages of growth. The mature plants were mid-tall with weak to mid-strong culms and with the upper peduncles hairy. The kernels were red, mid-sized to large, mid-long to long, slightly shriveled to medium plump, and somewhat resembled rye. The fertility was low to medium, the set of seed being comparable to that of the amphidiploid reported by Meister (*3*).

A small sample of seed of one of the Saratov selections was obtained [4] for seeding at Moscow, Idaho, in 1932 and 1933. This amphidiploid was more vigorous than the one obtained from Arlington farm. At maturity the spikes contained many empty florets.

Root tips from the Arlington farm amphidiploid were obtained by the writer from plants grown in the greenhouse. These were embedded in paraffin and taken to the University of Idaho, where the

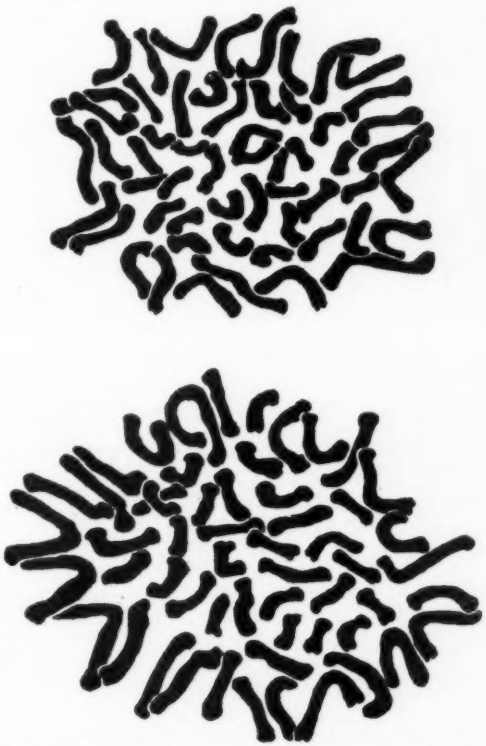

FIGURE 1.—Camera-lucida drawings showing two somatic metaphase plates of F_5 Purplestraw wheat × Abruzzes rye amphidiploid. ($2n=56$.) × about 1,375.

cytological study of the material was made in 1931. S. Navashin's chrom-acetic-formalin was used as the killing and fixing agent and Heidenhain's haematoxylin as the stain.

The chromosome number of the somatic cells was found to be 56. Counts were made on 6 cells; 5 had 56 chromosomes, and 1 had 58. The general shape and comparative size of these chromosomes are shown in figure 1. It was not possible to distinguish between the wheat and the rye somatic chromosomes.

In 1933 pollen mother cells of the amphidiploid were examined by Belling's aceto-carmine method. Counts were made on a number of

[4] Through the courtesy of E. F. Gaines, cerealist, of the Washington Agricultural Experiment Station.

different cells at first metaphase, and all were found to contain 28 chromosome pairs. Three polar views of first metaphase plates are shown in figure 2. The chromosome number indicated thus agrees with that found in the root tips.

Certain chromosome type differences were noted in the first metaphase of some pollen mother cells, whereas none was found in the somatic cells. In countable cells a number of large bent chromosomes usually were found scattered along the edges or outside of the more or less well-defined, centrally located group of typical bivalent chromosomes. Up to seven of these large, light-staining border chromosomes were found in some cells and they probably were those of rye. It is assumed in the following discussion that the large chromosomes were those of rye and the smaller chromosomes were those of wheat. In previous work with wheat × rye hybrids this tendency for the rye chromosomes to retreat to the edge or outside of the main group had been observed (*1*). This behavior apparently is due to incompatibility between the two sets of chromosomes as a result of their genetic differences. Figure 2, *C*, shows seven large light-staining bent-border chromosomes. The two large straight ones at the right represent wheat chromosomes, the ends of which evidently had been pressed flat by the cover glass. Figure 2, *A*, *B*, and *C*, shows large bent-border chromosomes in varying numbers.

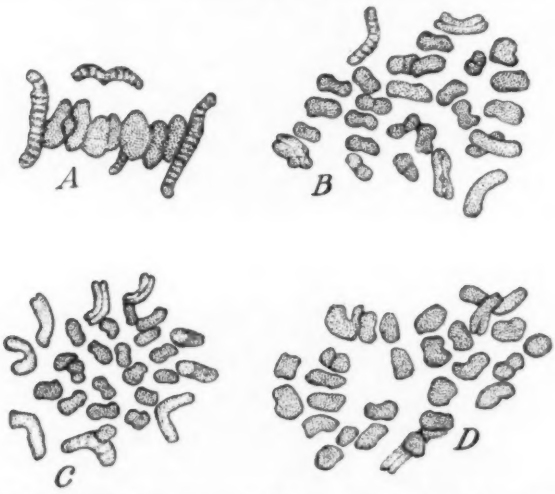

FIGURE 2.—Camera-lucida drawings of 4 meiotic-division metaphase plates of F_3 Purplestraw wheat × Abruzzes rye amphidiploid. (× about 1,000.) *A*, Equatorial view of first metaphase plate showing typical arrangement of the wheat bivalents, with the large rye chromosomes (here banded) at the edges or outside of the wheat group. *B*, Polar view of heterotypic metaphase plate. Note the 6 large chromosomes at the borders. (n=21 II W+7 II R.) *C*, Polar view of heterotypic metaphase plate. Note the 7 large bent-border chromosomes. (n=21 II W+7 II R.) *D*, Polar view of heterotypic metaphase plate. Note 3 or 4 large bent chromosomes at the plate border. (n=28 II.)

The wheat bivalents were somewhat smaller than the rye bivalents, usually more or less constricted at or near the middle, sometimes with a faint longitudinal line indicating the line of union of the paired chromosomes. They also stained more deeply. Some of the rye

bivalents were shaped like those of the wheat but were larger and absorbed the stain less readily; others were large and curved, sometimes showing the heterotypic (or homeotypic) split and were also definitely lighter staining. These chromosome types are shown in figure 2, *B*, *C*, and *D*.

Some of the large lighter stained border chromosomes were banded transversely. These bands were dark red and opaque in contrast to the lighter red areas between them. One banded chromosome is shown in figure 2, *B*, and another in *C*. Other banded chromosomes were present in *C* but are not shown. Figure 2, *A*, shows an equatorial view of a metaphase plate having 4 of the large border chromosomes, all banded, 1 of which is outside the equatorial plate.

DISCUSSION

The differences noted in staining reaction between the heterotypic chromosomes of wheat and rye may be explained partly by variable stages of development in the two types and partly by variations in composition. The differences observed among the rye chromosomes in some cells might result from variations in the rye chromosomes themselves, i. e., those possessing the greatest affinity for wheat chromosomes might be expected to resemble wheat most closely in staining reaction.

Sax (*7*) presents a reproduction of a photomicrograph of meiotic chromosomes of *Secale cereale* (his fig. 1), which shows the transversely barred or "banded" appearance seen by the writer in what he believed to be the rye chromosomes of the Arlington wheat-rye amphidiploid. The stage at which this condition may be seen presumably is the late first prophase of the heterotypic division.

Previous experiments with interspecific crosses in plants have shown that somatic gametes may be produced, as was first demonstrated by Rosenberg (*6*) in *Hieracium* sp. The occurrence of somatic gametes in F_1 wheat $\times$ rye hybrids was shown by the writer (*1*) in the production of 49-chromosome plants upon backcrossing to the wheat parent. This means that some of the gametes (ovules) of the F_1 hybrid had 28 chromosomes. The probable cytogenetic origin of the Arlington Farm amphidiploid is suggested by its history. It was produced by crossing a wheat $\times$ rye $\times$ wheat plant with rye. It is reasonable to suppose that somatic gametes (ovules) also may be formed in a 49-chromosome plant, which when crossed with rye pollen will complete the diploid chromosome complement of the rye and thus produce an amphidiploid (zygote).

Judging by the proportion of somatic gametes (3 out of 10) found in F_1 wheat $\times$ rye hybrids which the writer (*1*) determined in the F_1 backcross to wheat, it is possible that wheat $\times$ rye $\times$ wheat somatic gametes also may occur not too infrequently, so that the production of amphidiploids of the wheat-rye type would be greatly facilitated by crossing the backcross to one parent with the other parent.

SUMMARY

A constant wheat $\times$ rye hybrid, bred and selected by J. W. Taylor at the Arlington Experiment Farm, near Washington, D. C., in 1925, was found by the writer to be an amphidiploid. Fifty-six chromo-

somes were found in the root tips and 28 pairs in the pollen mother cells.

Wheat and rye chromosomes could not be distinguished in the somatic cells. In the pollen mother cells at first metaphase the rye bivalents were found to be larger than those of the wheat. In most cases they were less deeply stained, some were banded transversely, and all had a strong tendency to arrange themselves at the border or edge of the chromosome group.

This amphidiploid resulted from a cross of wheat × rye × wheat × rye, which makes it probable that somatic gametes were involved in its origin both in the F_1 wheat × rye hybrid and in the F_1 wheat × rye × wheat backcross.

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PHYTOPHTHORA ROT OF SUGAR BEET[1]

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INTRODUCTION

Phytophthora root rot of sugar beet (fig. 1), caused by *Phytophthora drechsleri* Tucker, was first noted in 1927 in the Cache Valley of northern Utah, causing most severe damage in an area about 18 miles in length between Smithfield and Wellsville. In 1928 and 1929 the disease was also found in the northern part of the Cache Valley in southern Idaho and near Ogden and Provo, Utah, and Rifle and Carbondale, Colo. During 1932 to 1934 the disease was found in the Sacramento Valley and delta section in California.[2] Tests made in 1927 and 1928 showed that the root size and sugar content of the sugar beet are reduced as a result of the disease. The present paper presents the results of a study of the disease, commenced in 1927.

FIGURE 1.—Phytophthora rot of sugar beet, showing typical infection of basal part of the taproot; collected in the Sacramento River delta section Oct. 10, 1933.

DISEASE FAVORED BY WET SOIL

In Utah the disease was confined mainly to the heavier, poorly drained Trenton clay soils in Cache Valley, although small areas of infection were found in low places in sandy loam soil. In the first field in which the disease was found, plants growing on a ridge elevated 1½ to 2 feet were free from the disease, while a high percentage of the plants in the rest of the field were diseased (fig. 2, *B*). The same condition was found in this field during the two following seasons (1928 and 1929).

In California the disease has been found only where irrigation water has accumulated and where the soil has been excessively wet at

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[2] TUCKER, C. M. THE DISTRIBUTION OF THE GENUS PHYTOPHTHORA. Mo. Agr. Expt. Sta. Research Bull. 184, 80 pp. 1933. (See p. 9.)

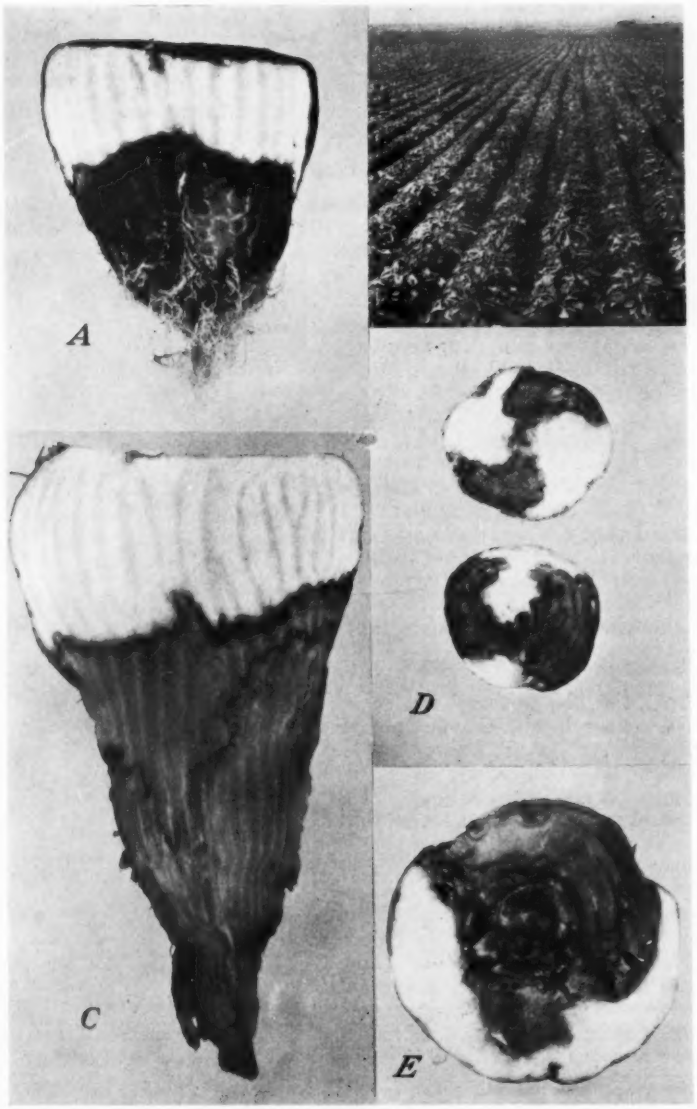


FIGURE 2.—*A*, Longitudinal section of an advanced case of Phytophthora rot, showing tassellike mass of vascular strands at basal end of the root; *B*, portion of field near Logan, Utah, October 1929, showing the severity of the disease, the leaves having wilted and most of the plants having been killed; *C*, longitudinal section of diseased root from field in Utah showing blackish-brown band of tissue at the sharply defined advancing edge of the citrine-drab, water-soaked infected tissue; *D*, cross sections of field-infected roots showing the black line at the irregular margin of the infected tissue; *E*, cross section showing cavities formed within the infected tissue and sunken nature of the external surface of the lesion.

some time during the season. It is generally recognized by growers as a trouble associated with excess soil moisture and is sometimes called wet rot or water rot.

In an irrigation experiment carried out in 1929 in the field in the Cache Valley where the disease was first found, three well-separated plots of 25 rows each were given different amounts of irrigation water. All plots received an irrigation 1 week after thinning, after which plot A received a light irrigation in furrows once every 2 weeks, plot B, every 3 weeks, and plot C, every 4 weeks. In plot A, the most heavily irrigated, 67 percent of the plants were infected, in plot B, 42, and in plot C, 15 percent. This indicates clearly that the more frequent irrigation favored infection, and that proper irrigation practice should control the disease.

EFFECT OF PHYTOPHTHORA ROT ON YIELD AND SUGAR CONTENT

Approximately 500 acres of beets were infected in varying degree in the southern half of Cache Valley in September 1927. In many fields, the number of healthy plants was so reduced that the yield did not defray the cost of harvesting. Losses ranged from a trace to 90 percent in individual fields. By count, 60 percent of the plants in one field presented unmistakable symptoms. Diseased plants occurred singly or in groups interspersed among healthy plants. Various stages of rotting of the root, accompanied by different degrees of wilting of the foliage, were exhibited, but for the most part advanced symptoms predominated.

More detailed surveys in 1928 and 1929 showed that in excess of 1,000 acres of land were more or less infested. Heavy losses were frequently met with on new land, plowed and planted for the first time. Many diseased roots, from which the fungus was isolated, were found in factory yards throughout the Cache Valley, 1 to 2 months after digging.

Preliminary tests in 1927 and 1928 showed that the disease reduced root size and sugar content. In August 1929 a representative area was selected for sampling in each of four fields in the Cache Valley. Healthy and diseased beets were dug, in as close proximity as possible. Diseased beets having more than half of the root rotted were discarded. The samples were weighed and analyzed in the laboratory of the sugar factory. The results are presented in table 1.

TABLE 1.—*Effect of Phytophthora rot on weight and sugar content of sugar beets in fields in Utah, Aug. 31, 1929* [1]

Location of field	Condition of beets	Beets	Average weight per beet	Sugar	Purity [2]
Logan:		*Number*	*Grams*	*Percent*	*Percent*
Field 1	Healthy	12	551.5	13.5	82.4
	Diseased	25	189.4	7.5	59.8
	do	25	146.5	5.3	47.8
Field 2	Healthy	12	432.9	15.8	84.8
	Diseased	25	132.4	9.9	61.2
	do	25	159.1	10.4	63.4
Field 3	Healthy	20	419.6	15.8	85.3
	Diseased	20	201.3	11.2	72.4
Smithfield	Healthy	20	544.3	17.0	84.1
	Diseased	30	266.5	11.6	72.1

[1] Analyses were made in the chemical laboratory of the Amalgamated Sugar Co. at Logan, Utah.
[2] Coefficient of purity is the ratio between the percentage of total solids and the percentage of sugar.

The average weight of the healthy beets was more than twice that of the diseased beets. The sugar in the diseased beets ranged from 5.3

to 11.6 percent, as compared with 13.5 to 17.0 percent in the healthy beets. The purity of the juice of the diseased beets ranged from 47.8 to 72.4 percent, as compared with 82.4 to 85.3 percent for the healthy beets.

Subsequent tests of the juice of samples of diseased beets collected during the remainder of the season from these and other fields gave even a greater reduction in sugar content and in purity so that at harvest the differences between healthy and diseased roots were even more striking.

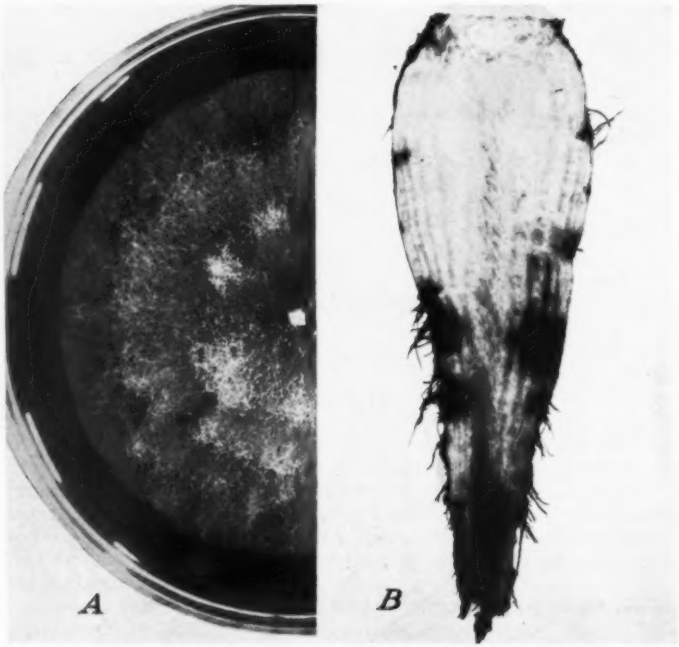


FIGURE 3.—*A*, Five-day-old culture of *Phytophthora drechsleri* on prune agar, pH 4.7, at 28° C.; *B*, longitudinal section of field-infected root showing lateral lesions, some at the bases of the rootlets.

SYMPTOMS OF THE DISEASE

In the early stages of the disease the leaves may wilt during the day and recover at night. Later, permanent wilting occurs and the plant is killed (fig. 2, *B*). Generally, infection occurs near the basal part of the root and the invasion progresses gradually upward involving all tissues of the root (fig. 1 and fig. 2, *C*, *E*). In roots inoculated in the laboratory, the affected tissues remain fairly firm and turgid (fig. 2, *C*), but under field conditions the affected parenchyma at the base of the root may soften and disintegrate, leaving only the vascular strands. This gives a frayed, tassellike, skeletonized appearance to the basal part of the fleshy taproot (fig. 2, *A*). Secondary or adventitious lateral roots often develop above the invaded region of the taproot and an ill-shaped, pronglike root is the result.

Often lesions first arise on the sides and sometimes near the upper part of the taproot (figs. 2, *D* and 3, *B*). Infrequently, infection occurs

at the tips or along the sides of the lateral roots or rootlets, with subsequent invasion of the taproot around the base of the infected lateral root or rootlet (fig. 3, *B*).

The lesions vary in size and shape and sometimes the entire root is involved. The predominant external color of the lesion ranges from the mummy brown to light seal brown of Ridgway,[3] tending toward blackish brown at the center.

When the root is cut, the infected tissues are found to be sharply delimited from the healthy tissues (fig. 2, *A*, *C*, *D*). Generally, color zones are visible in the lesion. The tissues first infected are deep brownish drab;[3] the central parts of the infected region are water-soaked and citrine drab to smoke gray, or buckthorn brown, or light buff to mummy brown; and the recently infected tissue at the advancing edge of the lesion as viewed on the cut surface is composed of a narrow band of blackish brown, sometimes separated from the healthy tissue by a narrow band of light buff (fig. 2, *C*, *D*). In the early stages the vascular bundles are conspicuous and light seal brown in color, but later this distinction may be lost. In the final stages the tissues are blackish brown (fig. 2, *A*). Small pockets or cavities are often formed (fig. 2, *E*) but are not filled with mycelium as in dry-rot canker caused by *Rhizoctonia solani* Kühn.

Microscopic examinations of stained sections of tissue from field-infected roots as well as roots inoculated in the laboratory show that the mycelium is, in general, intercellular, although certain cells are filled with mycelium. There was no evidence of any dissolution of the middle lamella.

ISOLATION OF THE CAUSAL FUNGUS

The causal fungus was readily isolated on prune agar, and usually pure cultures were obtained from tissue plantings from the advancing edge of the lesions. Isolations were made from more than 300 roots collected in 20 fields in Utah in 1927 and 1928. In not a single instance were other fungi or bacteria obtained (table 2). Good growth developed at 28° C. within 36 hours. The fungus was also isolated from infected lateral roots and lateral rootlets. Attempts to isolate the organism from healthy tissues of a diseased root, as well as from wilted and turgid petioles and leaves of diseased plants, gave negative results. The same fungus was isolated from diseased roots collected in subsequent years in Utah, and in 1932 in the Sutter basin and delta section in California.

TABLE 2.—*Isolations of Phytophthora drechsleri from diseased sugar-beet roots in Utah*

Location of field	Date of isolations	Roots	Roots yielding *P. drechsleri*	No growth
Logan:		*Number*	*Number*	*Number*
Field 1	Aug. 3, 1927	50	43	7
Field 2	Sept. 5, 1927	30	26	4
Field 3	Aug. 22, 1928	132	94	38
Field 4	Aug. 8, 1929	100	87	13
Smithfield	Aug. 23, 1928	50	40	10
Total		362	290	72

[3] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912

THE CAUSAL FUNGUS

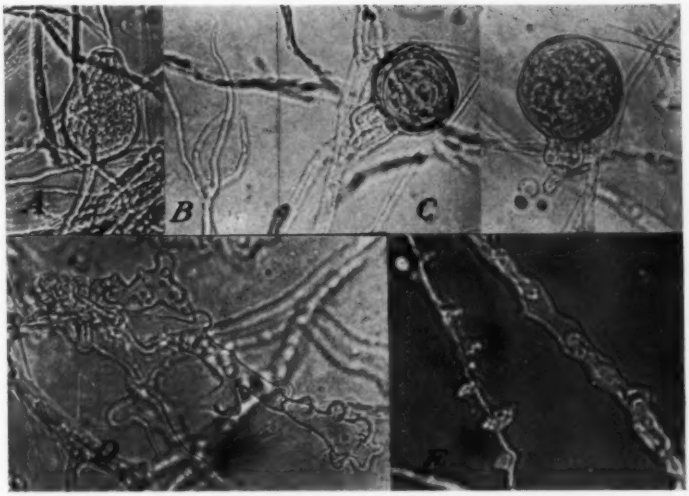
The causal organism was identified as *Phytophthora drechsleri* Tucker.[4] In young cultures on potato-dextrose agar the fungus developed a moderate amount of aerial mycellium with no reproductive organs, and on moist corn meal a white cottony mass of mycelium was present after 2 weeks. On prune agar the fungus produced a delicate, moderately dense mycelium with scant aerial growth (fig. 3, *A*). The fungus grew luxuriantly on oatmeal agar slopes, filling the space between the slope and the wall of the tube with a densely matted, tough, white mass of hyphae, but failed to develop reproductive bodies after 25 days at room temperature and produced only occasional oogonia after 2 months at 15° to 20° C. The hyphae were usually smooth and uniform in diameter, but occasionally they were gnarled and swollen, sometimes with tuberlike or vesicular growths, especially in old potato-dextrose agar cultures (pl. 1, *D*, *E*).

Oogonia with amphigynous antheridia developed sparingly in potato-dextrose agar cultures incubated 2 months at 15° to 20° C. They were thin-walled, hyaline to yellow or light brown, spheroidal with a narrow stalk completely enclosed by the antheridium (pl. 1, *C*). The oogonia varied in diameter from 27.2μ to 44μ, averaging 35.8μ. The antheridia were borne on slender hyphae smaller than the usual vegetative strands. They were generally 10μ to 14μ in diameter and often distorted by pressure on the oogonium. The antheridium formed a collar around the oogonial stalk and was closely oppressed to the inflated portion of the oogonium where it remained until the oogonium disintegrated (pl. 1, *C*).

The oospores developed singly in the oogonia. They were smooth, thick-walled, yellow to light brown, spherical, only partially filling the oogonial cavity (pl. 1, *C*). In old cultures the oogonial wall became crumpled and finally disintegrated, liberating the oospore. The oospores were 23.6μ to 35.2μ in diameter, averaging 28.6μ. Germination was not observed.

When tufts of mycelium from oatmeal-agar cultures 3 weeks old were transferred to Petri's solution sporangia developed after 2 days. The sporangia were hyaline to faintly yellow, thin-walled, obpyriform, acrogenous on slender sporangiophores, nonpapillate but provided with a hyaline lens-shaped thickening at the apical end, 24μ to 56μ by 22.8μ to 40μ, averaging 38.6μ by 26.7μ (pl. 1, *A*). Germination occurred by germ tubes or by the development of a variable number of motile zoospores, 10μ to 12μ in diameter, which were fully differentiated within the sporangium. The lenticular thickening in the apex of the sporangium was pushed out or dissolved and the zoospores escaped, soon became quiescent, rounded up, underwent thickening of the wall, and finally germinated by a slender germ tube, the usual type of development among species of *Phytophthora*. Following zoospore discharge, the sporangiophore often resumed growth, pushing through the base of the evacuated sporangium and growing out through the apical aperture (pl. 1, *B*). A new sporangium was often formed just beyond or occasionally within the old wall. Sometimes the process was repeated, leaving the walls of two evacuated sporangia enclosing the sporangiophore bearing a sporangium at the tip.

[4] TUCKER, C. M. TAXONOMY OF THE GENUS PHYTOPHTHORA DE BARY. Mo. Agr. Expt. Sta. Research Bull. 153, 208 pp., illus. 1931. (See p. 188.)



Phytophthora drechsleri, Tucker: *A*, Nonpapillate sporangium, with a refringent lens-shaped thickening at the apex, developed on hyphae from an oatmeal agar culture after 2 days in Petri's solution, × 430; *B*, an empty sporangium with the sporangiophore emerging from the orifice following resumed proliferation subsequent to discharge of the contents of the sporangium, × 430; *C*, at the left, oogonium, oospore, and antheridium and, at right, oogonium and antheridium, prior to the development of the oospore, the antheridium forming a collar around the narrow oogonial stalk, ×430; *D* and *E*, gnarled and tuberculate hyphae from a potato-dextrose agar culture 2 months old.

Tufts of mycelium transferred from oatmeal agar to a nonsterile soil suspension developed sporangiophores bearing typical nonpapillate sporangia, measuring 30μ to 48.8μ by 17.2μ to 36.8μ, the average dimensions being 36μ by 26μ, very similar to those developed in Petri's solution. Sporangia developed in 24 hours and more abundantly than in Petri's solution. Mehrlich,[5] using a similar procedure with hyphae of *Phytophthora cinnamomi* Rands grown in a nutrient broth, obtained papillate sporangia instead of the usual nonpapillate type. *P. drechsleri* developed sporangia sparingly in sterile beet-leaf decoction.

No well-differentiated chlamydospores were produced, but thin-walled, hyaline, frequently clustered, swollen vesicles of irregular size and shape usually appeared in old potato-dextrose agar cultures. These vesicles were not so abundant or so well developed as in cultures of *Phytophthora cinnamomi*.

The amphigynous antheridia and nonpapillate sporangia permit placing the fungus in the group of species which includes *Phytophthora erythroseptica* Peth., *P. cryptogea* Peth. and Laff., *P. richardiae* Buism., *P. cinnamomi* Rands, *P. cambivora* (Petri) Buism., and *P. drechsleri* Tuck. One of the authors [6] has shown that of this group *P. drechsleri* alone will grow at 35° C., and Leonian [7] reported growth at 37°. The beet-root fungus grows well at the former temperature. In morphological characters the fungus agrees quite closely with the description of *P. drechsleri*,[8] and in general growth characters cannot be distinguished from the type culture. Leonian indicated that *P. drechsleri* would be merged with *P. palmivora* Butl. should the genus be reduced to the three species he considers valid; however, he recognized differences in that *P. drechsleri* fails to form chlamydospores and produces nonpapillate sporangia while *P. palmivora* develops chlamydospores and papillate sporangia. The resumed growth of the sporangiophores through evacuated sporangia frequent in *P. drechsleri* and the other species here grouped with it has not been observed in *P. palmivora* and may be considered a rather important morphological character tending to warrant the continued separation of the two species. Drechsler [9] observed nonpapillate sporangia and resumption of growth of sporangiophores through empty sporangia in *P. megasperma* Drechsler. His report and the observations of the writers on this species indicate that it may be separated from the group of species including *P. drechsleri* by its early and profuse production of oogonia and oospores in culture, and by its numerous paragynous antheridia. Amphigynous antheridia have been reported for *P. drechsleri* and the species here grouped with it. The observations of the present writers on cultures of these species show that paragynous antheridia are rarely, if ever, present.

The only previous record of *Phytophthora drechsleri* is that of the type [10] isolated by Drechsler from a rotting potato tuber from Idaho. The isolation of this species from sugar beets in Utah and California extends its host and geographic distribution. Transfers of the cultures studied have been deposited in the Centraalbureau voor Schimmelcultures.

[5] MEHRLICH, F. P. NONSTERILE SOIL LEACHATE STIMULATING TO ZOOSPORANGIA PRODUCTION BY PHYTOPHTHORA SP. (Abstract) Phytopathology 24: 1139–1140. 1934. (Phytopath. note) 25: 432–435. 1935.

[6] TUCKER, C. M. See p. 119 of citation mentioned in footnote 4.

[7] LEONIAN, L. B. IDENTIFICATION OF PHYTOPHTHORA SPECIES. W. Va. Agr. Expt. Sta. Bull. 262, 36 pp., illus. 1934.

[8] TUCKER, C. M. See footnote 4.

[9] DRECHSLER, C. A CROWN-ROT OF HOLLYHOCKS CAUSED BY PHYTOPHTHORA MEGASPERMA N. SP. Jour. Wash. Acad. Sci. 21: 513–526; illus. 1931.

[10] TUCKER, C. M. See footnote 4.

TEMPERATURE RELATIONS OF THE FUNGUS IN CULTURE

The relation of temperature to growth of the mycelium was determined for two cultures, one from Utah and one from California. For this purpose, large test tubes 20 cm in length, provided with a dam at the open end, made by heating the glass and indenting one side, were used. Prune agar (15 cc) of pH 4.7 was placed in each tube and allowed to solidify with the tube in a horizontal position. The dam prevented the escape of the melted agar.

These tubes were inoculated near the mouth with small squares of prune agar with mycelium from Petri-dish cultures 5 days old. After an initial exposure at room temperature (20° to 23° C.) for 24 hours, the position of the advancing edge of the colony was marked with a wax pencil on each tube, and three tubes of each culture were placed in a horizontal position in thermostatically controlled, electric incubators at 3°, 8°, 12°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, 37°, and 40°. The daily growth of the mycelium beyond the wax-pencil mark was measured.

There was no marked difference between the Utah and California cultures. No growth occurred at 3°, or at 37° or 40° C. The average daily growth at 8° was 2.3 mm; at 12°, 5 mm; at 16°, 6.6 mm; at 19°, 7.3 mm; at 22°, 7.7 mm; at 25°, 9.6 mm; at 28°, 10.0 mm; at 31°, 10.0 mm; and at 34°, 8.7 mm. In another test on corn-meal agar in Petri dishes, the average diameter of the colony after 96 hours was 44 mm at 20°, 48 mm at 25°, 54 mm at 30°, and 49 mm at 35°. The lowest temperature for growth, therefore, is between 3° and 8°, the optimum, 28° to 31°, and the maximum between 35° and 37°. These characteristics, particularly the tolerance of high temperatures, are in agreement with those reported by Tucker [11] for the type culture.

RELATION OF HYDROGEN-ION CONCENTRATION TO GROWTH OF THE FUNGUS

Prune agar, adjusted to different pH values between pH 3.6 and pH 8.4 and placed in the large test tubes as previously described, was used to determine the relation of hydrogen-ion concentration to the growth of the fungus. The cultures were incubated at 28° C., and the daily growth increments over a period of 12 days were measured.

Good growth occurred at all the pH values tested, varying from 7.6 mm per day to 12.7 mm per day, with no indication that the limits of hydrogen-ion or hydroxyl-ion tolerance were being approached. The daily rate of growth at pH 3.6 was 8.3 mm; at 3.9, 9.6 mm; at 4.2, 12.7 mm; at 4.5, 9.9 mm; at 4.8, 10.0 mm; at 5.1, 10.0 mm; at 5.4, 9.7 mm; at 5.7, 10.2 mm; at 6.0, 9.9 mm; at 6.3, 10.2 mm; at 6.6, 10.5 mm; at 6.9, 9.8 mm; at 7.2, 10.0 mm; at 7.5, 8.3 mm; at 7.8, 7.6 mm; at 8.1, 10.6 mm; and at 8.4, 10.7 mm.

INFECTION OBTAINED ON WOUNDED AND UNWOUNDED PLANTS IN THE FIELD

For preliminary field tests in Utah in 1927 healthy vigorous plants were selected, and the soil was carefully excavated from one side of each root on August 15. The exposed side of the root was wiped with wet cotton and then with alcohol. In one series of inoculations, punc-

[11] TUCKER, C. M. See p. 189 of citation mentioned in footnote 4.

tures were made with a flamed needle well below the middle of the fleshy taproot, and mycelium from cultures on prune agar or sterilized oats was placed in contact with the injured periderm and the soil replaced. In a second series the inoculum was applied to the unwounded surface of the root. Control plants were treated similarly in each series except that sterile culture medium was substituted for the inoculum.

All plots were given two light irrigations between the time of inoculating and harvesting. Marked wilting developed on most of the inoculated plants within 30 days, and when the beets were dug on September 20, 82 percent of the 160 roots inoculated with wounds were infected and 41 percent of the roots inoculated without wounding were infected. The control plants remained healthy. The infected roots bore typical lesions, those on the wound-inoculated roots being the larger. The fungus was reisolated from a large number of the infected roots and in inoculations made on 10 plants on September 28 again proved pathogenic. Additional inoculation tests were made in Utah in the summers of 1928 and 1929. The results together with those of 1927, are summarized in table 3.

On August 8, 1929, healthy beets in the field were inoculated with small blocks of naturally infected beet tissue. Of 20 beets wounded by needle punctures, 17 were infected when examined on September 22, and of 20 beets inoculated without wounding, 13 were infected. The 10 control plants for each method remained healthy. The fungus was reisolated from the infected beets (table 3).

On August 8, 1934, plants were inoculated in the field at Clarksburg, Calif., by placing mycelium grown on cracked wheat in the soil alongside the unwounded roots. The beets were irrigated several times and were dug on September 20. Infection had occurred, and the fungus was reisolated.

INFECTION OBTAINED ON WOUNDED AND UNWOUNDED ROOTS IN THE LABORATORY

In Utah, in 1927, cultures of the fungus were used in crown inoculations of healthy topped beets. All beets were washed in tap water, treated for 10 minutes in a 1-to-1,000 solution of mercuric chloride, and rinsed in three changes of sterile distilled water. A thin slice of crown tissue was removed with a flamed knife to expose fresh tissues free from cork cells. The inoculum, mycelium from 8-day cultures on prune agar, was placed on the cut surface and covered with moist absorbent cotton held in position by means of adhesive tape. All beets were placed in individual glassine bags and stored in a root cellar at a temperature ranging from 5° to 10° C. After 35 days, 24 of the 28 inoculated beets were found to be infected, as proved by reisolations, and the amount of decay in individual beets ranged from 15 to 70 percent by volume. The 10 controls treated with sterile prune agar were not infected.

Inoculation tests were made on the unwounded surface of sugar beets in the laboratory at Berkeley in January 1933, with one Utah and three California cultures of the fungus. Beets were thoroughly washed in tap water, and 95-percent alcohol was applied to the periderm where the inoculations were to be made. Mycelium grown on prune agar was inserted in two wells in each root made by removing

small cubes of tissue with a flamed scalpel, and covering with adhesive tape. Controls were treated with sterile prune agar. All were incubated in moist chambers at laboratory temperatures (20° to 24° C.) for 20 to 45 days. For moist chambers, use was made of 5-gallon tin cans with pie tins for covers; each can was provided with a wire-mesh platform to prevent contact of the roots with the water in the bottom of the can. Of the 44 roots inoculated with the California cultures, 39 were infected, and of the 39 inoculated with the Utah culture 30 were infected. The fungus was reisolated from all of the infected roots. None of the 21 controls became infected. The cultures showed no difference in virulence.

TABLE 3.—*Results of inoculations on sugar beets with Phytophthora drechsleri in Utah, 1927–29*

Type of inoculum	Method of inoculation	Beets inoculated	Beets infected	Beets from which *P. drechsleri* was reisolated
		Number	*Number*	*Number*
Mycelium on agar	Needle	170	144	69
	No injury	130	62	73
Mycelium on oats	Needle	80	60	40
	No injury	40	16	34
Infected beet tissues	Needle	20	17	9
	No injury	20	13	10
Control, sterile agar	Needle	105	0	--------
	No injury	105	0	--------
Control; sterile oats	Needle	60	0	--------
	No injury	60	0	--------
Control; healthy beet tissues	Needle	10	0	--------
	No injury	10	0	--------

Unwounded roots were also successfully inoculated. On March 14, 1933, eight roots harvested in November and kept in cold storage were inoculated by placing a half-inch square from a prune-agar culture on a clean, sterilized but unwounded portion of the root surface. The roots were incubated in moist chambers at room temperature and within 2 weeks 5 of the 8 inoculated beets were typically diseased and the fungus was reisolated. The controls, on which sterile agar had been placed, were not infected.

BEET SEEDLINGS SUSCEPTIBLE TO INFECTION

No infection of sugar-beet seedlings by *Phytophthora drechsleri* has been observed under field conditions. Many diseased seedlings have been cultured, but this fungus has never been obtained. Presumably soil temperatures during the seedling stage of this crop are below the optimum for this fungus.

Laboratory tests showed, however, that seedlings were readily infected at higher temperatures. For these tests, small paraffined paper cups, 3 inches deep, were filled with sterilized river sand. Prune-agar cultures of the fungus were incorporated with the surface soil in the cups and 10 surface-sterilized, sugar-beet seed balls were planted in each cup. All cups were irrigated uniformly with sterile, distilled water and placed in incubators at constant temperatures of 25°, 28°, 31°, and 34° C. The seed balls failed to germinate at 34°.

Abundant infection of the seedlings occurred in 9 days at the other temperatures, as is shown in table 4. Infected seedlings exhibited damping-off symptoms, and the cortical tissues were water-soaked in appearance. Some seedlings attained a height of 1½ inches before being killed, but most of them were attacked in earlier stages of growth. Some infected seedlings barely emerged, and it was apparent that many died before emergence. The fungus was reisolated from practically all of the infected seedlings. Normal seedlings developed in the controls.

TABLE 4.—*Infection of sugar-beet seedlings grown in sterilized inoculated sand at constant temperatures*

Temperature (° C.)	Source of culture	Seedlings	Seedlings diseased	Temperature (° C.)	Source of culture	Seedlings	Seedlings diseased
		Number	*Number*			*Number*	*Number*
25	California	39	32	31	California	21	12
	Utah	27	25		Utah	11	6
	Controls	58	0		Controls	28	0
28	California	19	18				
	Utah	6	6				
	Controls	51	0				

PATHOGENICITY OF FUNGUS TO CERTAIN OTHER HOSTS

Tucker [12] found *Phytophthora drechsleri* pathogenic to wounded apple, eggplant fruits and tomato fruits, wounded eggplant, papaw, and tomato seedlings, and potato tubers. Wound inoculations have been successful on the roots of garden beets, carrots, turnips, parsnips, and potatoes. The identity of the fungus was confirmed by reisolations. In garden beets the diseased tissues were black and firm, in carrots water-soaked and moderately firm except in advanced stages, and in parsnips and turnips gray, water-soaked, and soft and mushy after 7 days. In potato tubers the infected tissues were softened and rubbery after 5 days, and cut surfaces when exposed to the air developed a light pink discoloration within 15 minutes which darkened within an hour and changed to black. A definite line separated diseased from healthy tissues.

Wound inoculations with the fungus were successful in watermelons and citrons. Large brown lesions developed on the surface within 48 hours at room temperature, and within 10 days the fruits had softened and were almost completely rotted.

Sixteen unwounded ripe tomato fruits were inoculated by placing small squares of an agar culture on the skin at the blossom end and holding them in glass, moist chambers at room temperature. All became infected, some in 6 days, and showed softened, water-soaked lesions, with irregular margins.

The pathogenicity of the fungus was also tested by inoculations on unwounded apple, eggplant, summer squash, green tomato, and green pepper fruits. The apples and green tomatoes were not infected. Eggplant fruits were readily invaded, and after 5 days chestnut-brown lesions 5 cm in diameter developed. The tissues were softened and discolored to a depth of 1 to 1½ inches. Two of the twelve green peppers inoculated and 7 of the 16 summer squashes were infected.

[12] TUCKER, C. M. See pp. 69 and 189 of citation mentioned in footnote 4.

Wound inoculations were made on apples, summer squashes, green tomatoes, and green peppers. After surface sterilization, each fruit was punctured 10 times with a flamed needle in an area one-half inch in diameter. A small square of agar from a culture was then placed on the punctured area. Three days later all the apple fruits were slightly invaded, the summer squashes were half decayed, the peppers were all infected, and the green tomatoes were almost completely softened. All controls were healthy at the end of the test period.

SUMMARY

Phytophthora rot of the sugar beet has been found in Utah, Idaho, Colorado, and California in poorly drained soils or areas where irrigation water has accumulated. Infection is favored by excessively wet soil. Yield and sugar content are greatly reduced.

The basal end of the taproot is usually rotted, although infection may occur along the side of the taproot, especially where rootlets are attached. In the field the disease causes wilting of the leaves during the day and ultimate death of the plant. In the early stages the infected root tissues as seen on the cut surface are water-soaked and light brown, with a thin blackish-brown zone at the advancing face of the lesion. Later the parenchyma tissues may disintegrate and leave a tuft of fibers at the tip of the root.

The disease is caused by *Phytophthora drechsleri* Tucker, of which a description is given. The fungus grows at temperatures ranging from 8° to 35° C., with its optimum at 28° to 31°. It grows well throughout a wide pH range.

With mycelium as inoculum, infection was readily obtained on both wounded and unwounded roots of plants in the field and also on harvested roots in the laboratory.

The fungus caused damping-off of sugar-beet seedlings grown in inoculated soil at laboratory temperatures.

In the laboratory wound inoculations with mycelium were successful on the roots of garden beet, carrot, turnip, and parsnip, on potato tubers, and on apples, summer squash fruits, green peppers, and green tomatoes. Inoculations without wounds were successful on eggplant fruits, green peppers, and summer squash fruits, but not on apples or green tomatoes.

HISTOLOGICAL STUDY OF TISSUES FROM GREENHOUSE TOMATOES AFFECTED BY BLOTCHY RIPENING [1]

By H. L. SEATON, *research assistant*, and G. F. GRAY, *assistant*, *Section of Horticulture, Michigan Agricultural Experiment Station* [2]

INTRODUCTION

The appearance and quality of greenhouse tomatoes (*Lycopersicon esculentum*) maturing in late spring and early summer are often impaired by red and colorless markings, commonly called blotchy ripening. In some cases the percentage of irregularly ripened fruits is less than 1 percent, while in others it may be as high as 50 percent. Investigations as to the nature of the disorder and methods for its control are being conducted at the Michigan Agricultural Experiment Station. This paper presents, as a part of these investigations, the results of a histological study of the tissues from blotchy-ripened fruits.

EXTERNAL APPEARANCE OF BLOTCHY RIPENING

Blotchy ripening is primarily a disorder of the spring crop of greenhouse tomatoes maturing in May, June, and July and is of little consequence to the fall crop maturing from October to January. Throughout the North Central States it rarely occurs under field conditions, although a few fruits showing all the characteristics of the disorder were observed during the period of the severe drought of 1934.

In affected fruits areas of the outer wall [3] (pericarp) fail to develop and color normally. The individual blotch may be relatively large, involving from 25 to 50 percent of the entire surface of the fruit, or may be so small that it is not readily distinguishable. A fruit may show only one small blotch or several large blotches; large and small blotches may be formed on the same specimen. No sharp line of demarcation separates the green or white areas from the normal red portions; they merge gradually. Though these areas may appear on any portion of the fruit, they generally radiate from the pedicel attachment. Blotchy fruits in which all portions of the fruits are involved are shown in plate 1. As the fruits approach maturity these areas remain hard and green; and as ripening proceeds further, they assume a waxy or glassy appearance which may be accompanied by a sunken or pitted condition.

The vascular bundles lying beneath these clear, glassy blotches invariably appear to be brown or black and necrotic. This condition may be observed through the epidermis in many fruits. An affected area may be confined to one of the furrows formed above the interlocular walls of the fruit; such areas have been frequently observed immediately above the main bundle of a carpel. Transverse cuts made through a blotchy fruit (pl. 2, *A*) show that only the outer wall is affected. Usually there are small discolored regions in the center

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[2] The writers are grateful to F. C. Bradford for helpful suggestions and assistance as to the methods employed and in the preparation of the manuscript.
[3] The term "outer wall" as used in this paper does not include the partitions between the locules.

of the blotch which have the appearance of necrotic vascular bundles. In severe cases cavities may be associated with the affected tissues. The discolored portions are found only in the distinct blotches and do not occur in the normally ripened portions. Longitudinal cuts (pl. 2, *B*) reveal the apparently necrotic bundles and associated tissues even more strikingly than do the transverse ones. As shown in the longitudinal cuts this appearance of broken-down tissues is confined entirely to the blotchy areas.

The disorder is apparently restricted to practically mature fruits. Fruits in all stages of development have been dissected and examined for evidences of blotchiness. No immature fruit has shown any detectable signs of developing into blotchy fruit. The first evidences of blotchiness have been observed only after the fruit begins to develop color, which is usually from 2 to 5 days before it is ready to harvest.

LITERATURE REVIEW

Bewley and White (*2*)[4] attempted to dissect tomato fruits so as to expose the vascular system, but "owing to the delicate nature of the individual strands", abandoned this method and adopted clearing methods. They carefully removed the skin of a half-ripe fruit, scraped away the superficial tissue, and exposed the external portion of the vascular system. They presented drawings showing that the veins radiate from the stem end, and that in the proximal half of the fruit there are few cross connections, while in the distal half the veins anastomose to form an intricate network. By clearing a fruit thus prepared in xylol following alcohol they obtained "a good idea of the vascular system of the entire fruit."

Their examinations revealed certain differences between the vascular bundles of blotchy fruits and those of healthy fruits. "The bundles of blotchy fruits seemed somewhat thicker than those of healthy fruits, owing to the spongy nature of the cells immediately surrounding them. Necrosis of the bundles frequently occurs" (*2*, *p. 325*). Drawings are shown of necrotic bundles appearing beneath distinct blotches. In another illustration they showed an affected bundle with an adjacent group of "corky cells" and stated that "such cells occur frequently in blotchy fruits." By means of a hand lens they observed "the presence of gaps or canals in the parenchyma bordering the necrotic bundles." The walls of these cells were brown and necrotic. Although no mention is made of the fact, their drawings indicate that plugging of the tracheae accompanies the necrosis.

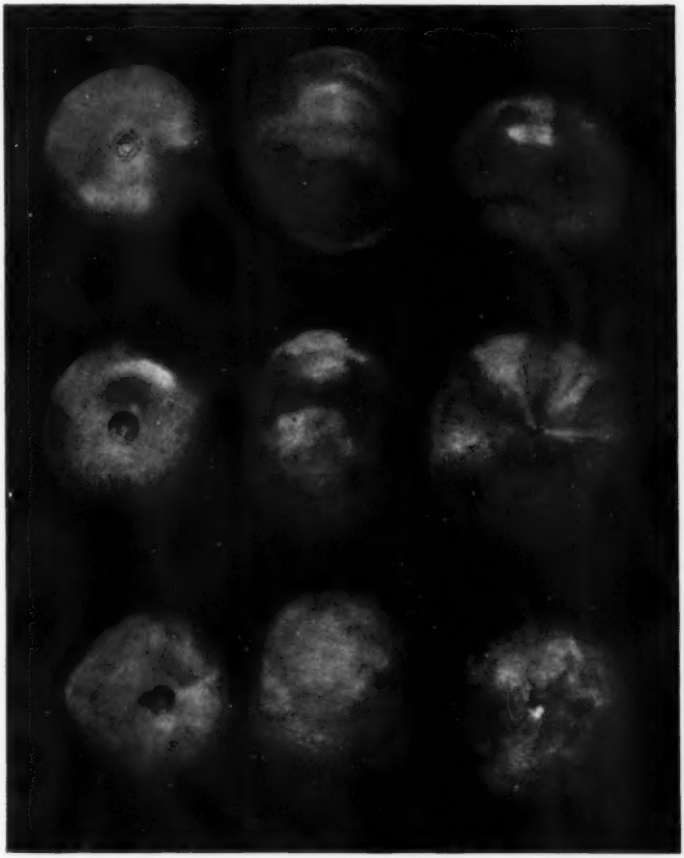
Bewley and White (*2*, *p. 337*) from their results over a 5-year period (1921–25) concluded that "blotchy ripening of tomato fruits is the result of malnutrition in respect of potash and nitrogen", and "may be reduced to less than 1 percent. by suitable applications of sulphate of potash and sulphate of ammonia." However, they did not attempt to connect "the necrosis of the bundles" with these nutrient deficiencies.

Seaton's investigations of the disorder [5] (*12*) indicate that blotchy ripening is not a nutritional disorder arising from soil conditions. He has advanced the hypothesis that the withdrawal of water from the

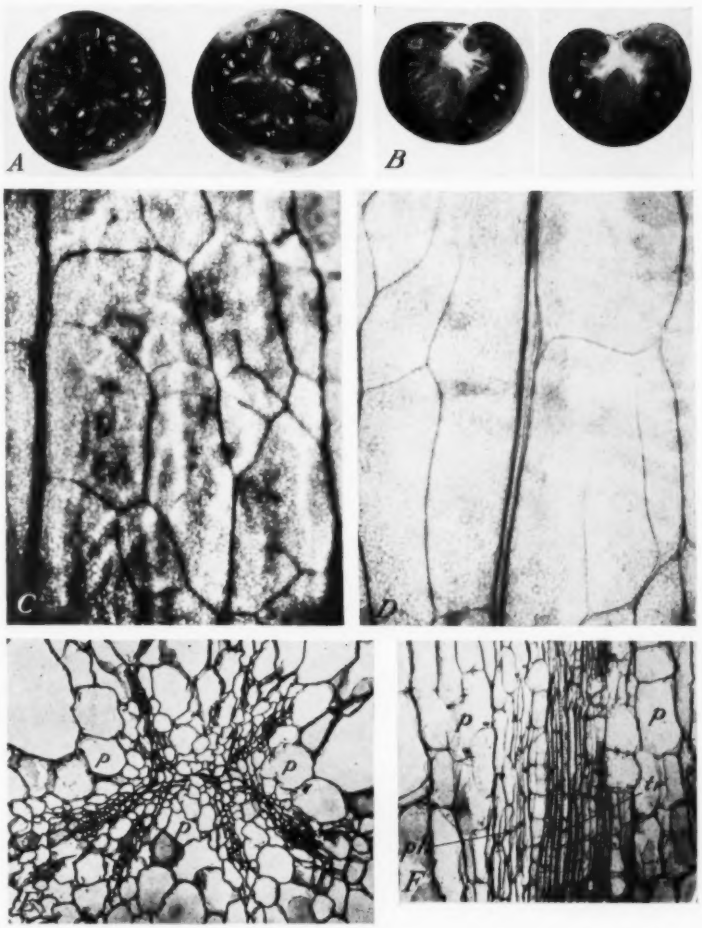
[4] Reference is made by number (italic) to Literature Cited, p. 224.

[5] SEATON, H. L. INVESTIGATIONS ON BLOTCHY RIPENING OF GREENHOUSE TOMATOES. 1933. (Unpublished thesis, Mich. State Col.).

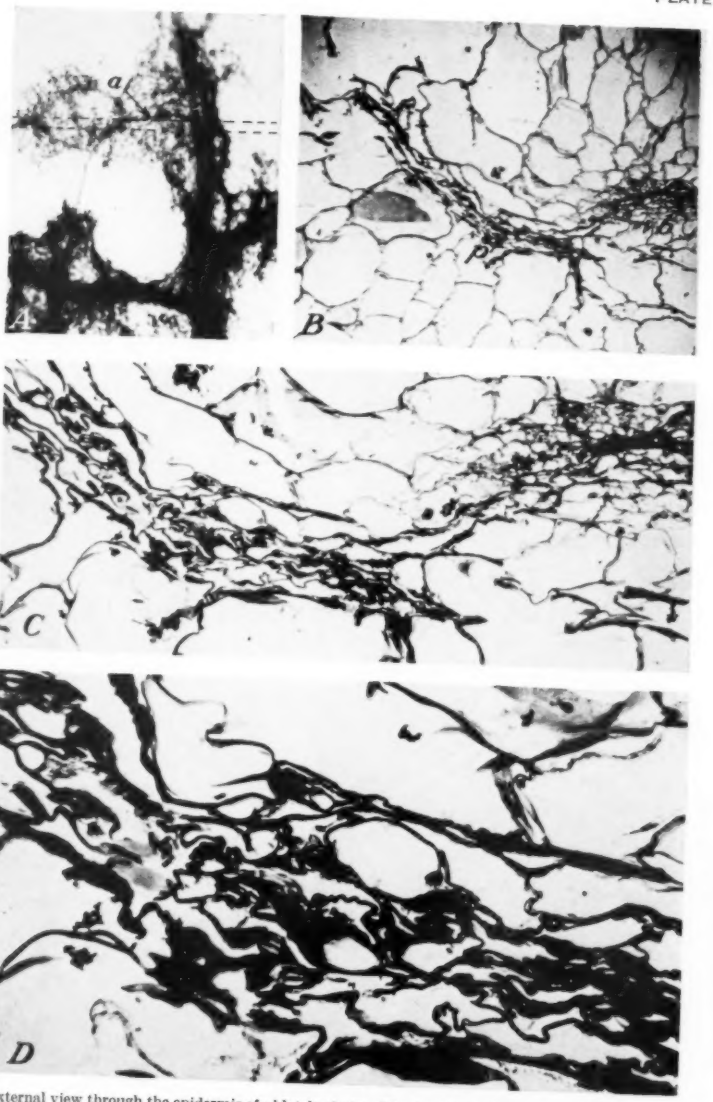
Greenhouse Tomatoes Affected by Blotchy Ripening PLATE 1



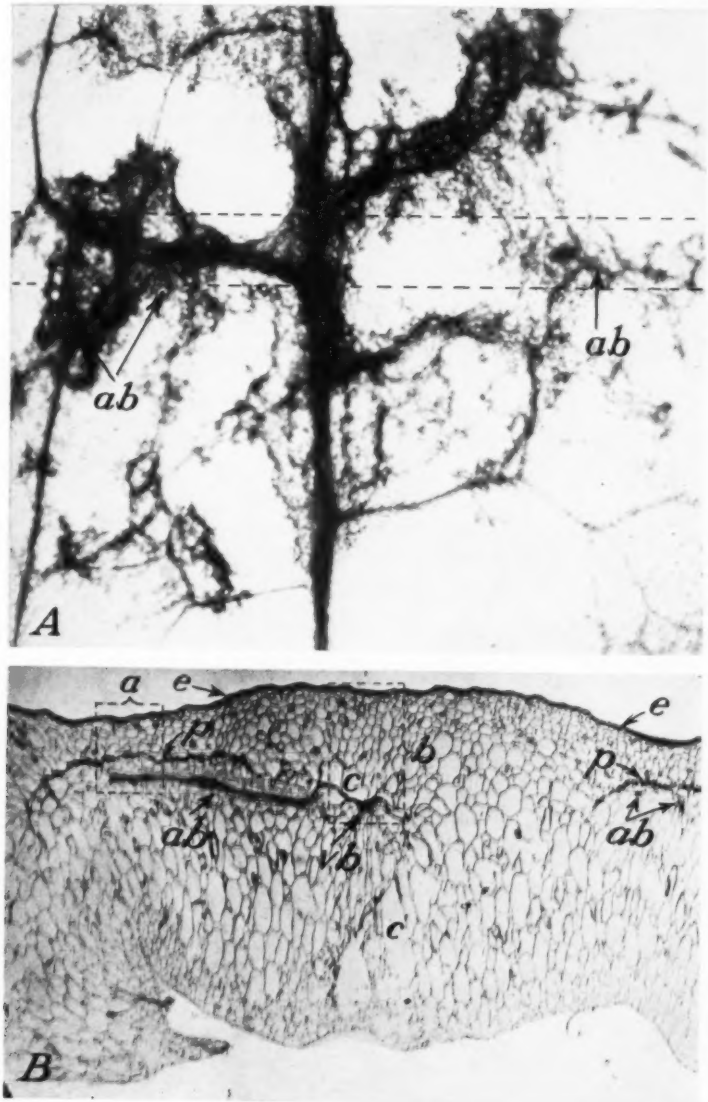
Tomatoes showing blotchy ripening. The glassy areas appear on all parts of the fruit but are less abundant in the stylar region. Some of the blotchy areas show a sunken or pitted condition, whereas dark, discolored tissues adjacent to the bundles appear in others.



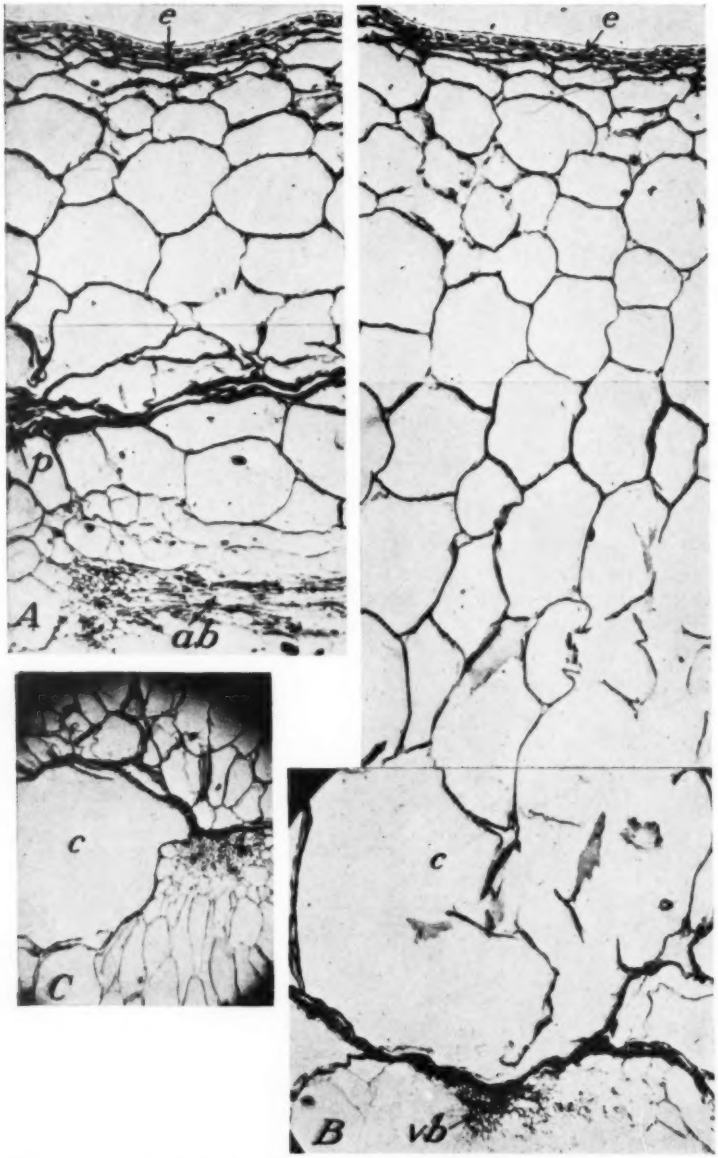
A, Blotchy ripened fruits cut transversely. Only the outer fruit wall is involved. The small dark areas in the blotches are affected tissues adjacent to the vessels. × ½. *B*, Fruits cut longitudinally to show the position of the affected tissues in the blotchy areas. No discolored tissues are apparent in the normal pericarp. × ½. *C*, A cleared section from a normal fruit wall near the stylar region. One large carpellary bundle and numerous small anastomosing veins are shown. × 5. *D*, Same as *C*, from an area near the stem end. The anastomosing veins are less numerous in this region. × 5. *E*, Transverse section of a large carpellary bundle from a normal pericarp. This bicollateral bundle is typical. Note the large parenchyma cells (*p*) of the vascular ray. × 125. *F*, Longitudinal section of a bundle from an unaffected fruit wall. The spiral thickened tracheae (*tr*), the adjacent phloem (*ph*), and the surrounding parenchyma (*p*) are shown. × 125.



A, External view through the epidermis of a blotchy fruit. The discolored tissues appear to surround and involve the large bundle and the anastomosing veins. The broken lines (*a*) indicate the position of the section shown in *B*. × 10. *B*, Transverse section from area indicated in *A*, *a*. Note the large vascular bundle (*vb*) and the broken down parenchyma tissue (*p*) which lies between the anastomosing bundle shown in *A* and the epidermis. × 50. *C*, Identical with *B*. The vessels of the bundle appear unaffected and the discolored areas are collapsed parenchyma. × 145. *D*, Identical with *C*. The distorted cell walls and cell contents which make up the dark areas are shown. × 450.



A, View through epidermis of a blotchy fruit taken in the region of a carpellary bundle. Normal areas may be seen among the discolored portions which surround the anastomosing bundles (*ab*). The broken lines indicate the position of the cross section shown in *B*. × 10. *B*, Transverse section of region indicated in *A*. Note the wavy appearance of the epidermis (*e*) with sunken portions above regions where the underlying parenchyma (*p*) has collapsed, while above the unaffected areas and where cavities (*c*) appear it is unaltered. The dark collapsed parenchyma tissues (*p*) are located between the epidermis (*e*) and the bundles (*ab* and *vb*). See plate 5 for details of the marked portions *a* and *b*. × 10.



A, Region indicated as *a* in plate 4, *B*. The epidermal cells (*e*) and the several underlying layers appear normal with the dark band resulting from the collapse of several layers of parenchyma (*p*) immediately above the anastomosing bundle (*ab*). The bundles show no evidences of necrosis or plugging, and apparently the cells subjected to break-down lie between the bundles and the epidermis. × 75. *B*, Region indicated as *b* in plate 4, *B*. Compare the thickness of this area with that shown in *A*, where a collapse of cells is apparent. The large cavity (*c*) seems to be made up of dismembered cell walls, some of which have collected around the vessels of the bundle. As in *A* the broken-down tissues are located between the epidermis (*e*) and the bundle (*vb*). × 75. *C*, Same bundle as shown in lower portion of *B*, but from a section taken above that shown in *B* The vessels are unaffected and the dark areas are made up of distorted walls and cell contents of the parenchyma which once filled the cavity (*c*). × 75.

fruits during periods of excessive transpiration, 3 to 5 days before the fruit ripens, results in the break-down of the tissues involved in the blotchy areas.

MATERIALS AND METHODS

The normal and blotchy-ripened fruits which furnished the material for this investigation were grown in the experiment station greenhouses at East Lansing in 1933 and 1934. Samples were collected each year in June and were about equally divided between fruits of the Grand Rapids variety and fourth- and fifth-generation hybrids between Ailsa Craig and Marglobe. In 1933 comparatively small portions of the outer fruit wall from distinct blotches were excised from freshly harvested material; but in 1934 much larger portions were taken, in some cases as much as one-fourth of the entire outer wall being used. The epidermis was removed from some samples and allowed to remain intact on others. In 1933 the material was killed and fixed in alcohol-formalin-acetic acid killing solution, embedded in paraffin, sectioned, and stained with Delafield's haematoxylin. In 1934 the samples were handled in the same manner, except that the large sections, after being cleared in xylol, were photographed with transmitted light in that liquid in an especially designed cell. After the gross photographs (pls. 2, *C* and *D*; 4, *A*; and 6, *A*), at a magnification of 5 and 10 diameters, were examined, the portions indicated in the plates were dissected from the cleared samples, embedded in paraffin, sectioned, and stained in the same manner as the 1933 material.

STRUCTURE AND APPEARANCE OF THE PERICARP

THE PERICARP FROM NORMAL FRUITS

When the outer fruit walls are cleared in xylol following alcohol, the vascular anatomy of the pericarp may be followed easily. Several large carpellary bundles radiate from the corky abscission layer, extend around the fruit, and converge again at the stylar scar. Relatively large branches from the main carpellary bundle are found in the furrows outside the interlocular walls and smaller anastomosing veins from these extend into these walls (pl. 2, *B*). In the outer carpellary walls other branches of the main bundle appear and extend with it to the stylar scar. In the pericarp near the pedicel attachment and extending to the region of the greatest diameter of the fruit few connecting veins are present between the main bundle and the large branches (pl. 2, *D*). However, from the region of the greatest diameter to the stylar scar the bundles anastomose freely and form an intricate network of connecting veins (pl. 2, *C*). Thus the areas between the bundles and veins are larger, and more of their parenchymatous cells are further removed from bundles in the lower half of the ovary wall than in the upper half. These observations are, in general, in agreement with those of Bewley and White (*2*) and the observations by Cooper (*4*) on the anatomy and development of the tomato flower.

Histological examinations of the pericarp from mature fruits (pls. 4, *B*; and 5, *A* and *B*) show that the outer epidermis is made up of small, heavily cutinized polygonal cells. The inner epidermis is more delicate than the outer and shows little or no cutinization. Only the

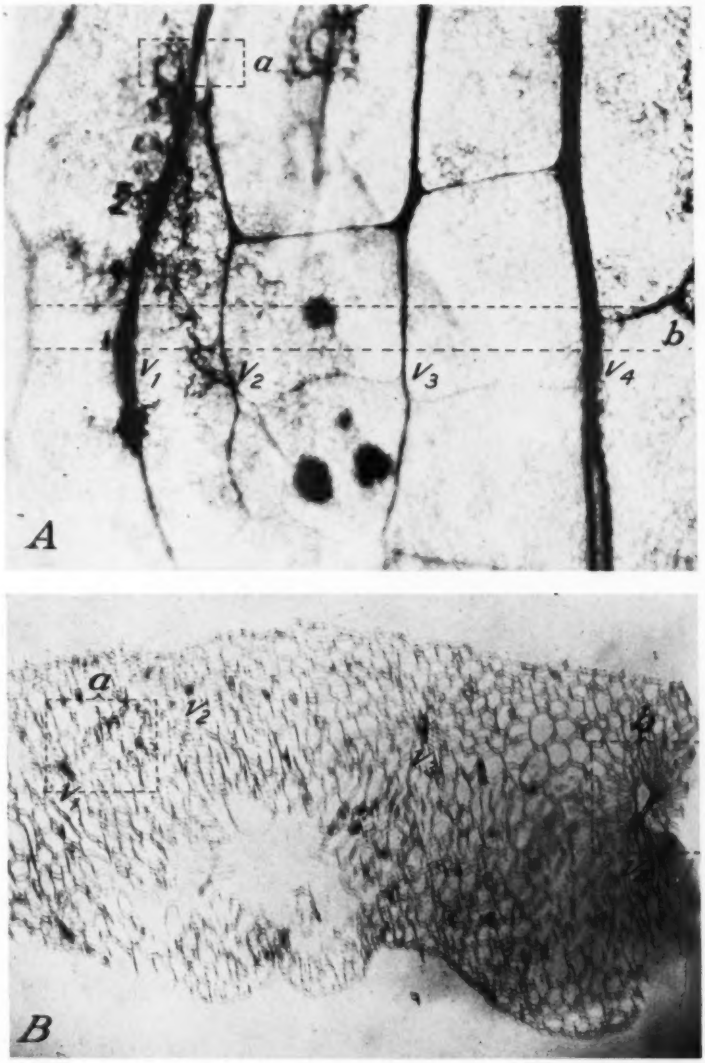
outer and the inner epidermis and the fleshy layer between the two are distinct. The several layers of parenchyma cells constituting the fleshy layer adjoining the epidermal layers are smaller and have slightly thicker walls than those of the center, where the cells are greatly extended and the walls are excessively thin and delicate at maturity. Intercellular spaces of any appreciable size are not present. Throughout the center of the fleshy layer, bicollateral vascular bundles, dissected with vascular rays of large, spongy parenchyma occur (pl. 2, *E*). In longitudinal section the tracheae of these bundles are seen to be the spirally thickened type (pl. 2, *F*).

THE PERICARP FROM BLOTCHY FRUITS

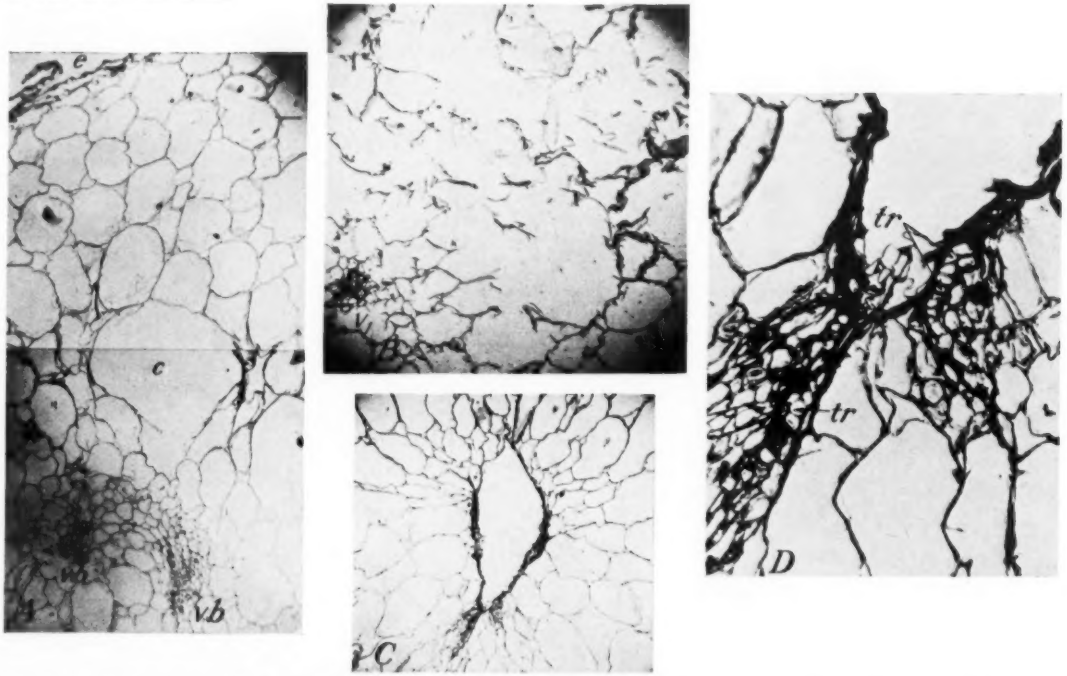
In cleared sections from blotchy fruits large dark areas attendant to the bundles and their branches are apparent, while less dense, slightly diffused areas of similarly affected tissues appear between the bundles (pls. 4, *A* and 6, *A*). Under low magnification the bundles in blotchy regions seem to be enlarged throughout the extent of the blotch and give the appearance of being necrotic and functionless, as Bewley and White (*2*) reported from their observations with a hand lens. However, when carefully prepared sections are examined under higher magnification, the actual conditions of the vascular elements and surrounding tissues appear quite different.

An external view through the epidermis of a blotchy fruit (pl. 3, *A*) illustrates the deceptive appearance of the necrotic and enlarged bundle and the anastomosing veins surrounded by affected tissue. In a transverse section of this same area (pl. 3, *B*) at higher magnification, the large bundle appears to the right of the field and the band of discolored tissue in the center is from immediately below the anastomosing bundle. The elements which appeared plugged at lower magnification are in reality open and unaffected, with some broken-down vascular ray parenchyma between them. The band of dense material, shown under high power (pl. 3, *C* and *D*), is clearly composed of collapsed parenchyma cell walls and the cell contents from the center of the fleshy layer of the ovary wall. The arrangement of the collapsed cells seems to indicate that they were subjected to mechanical stresses.

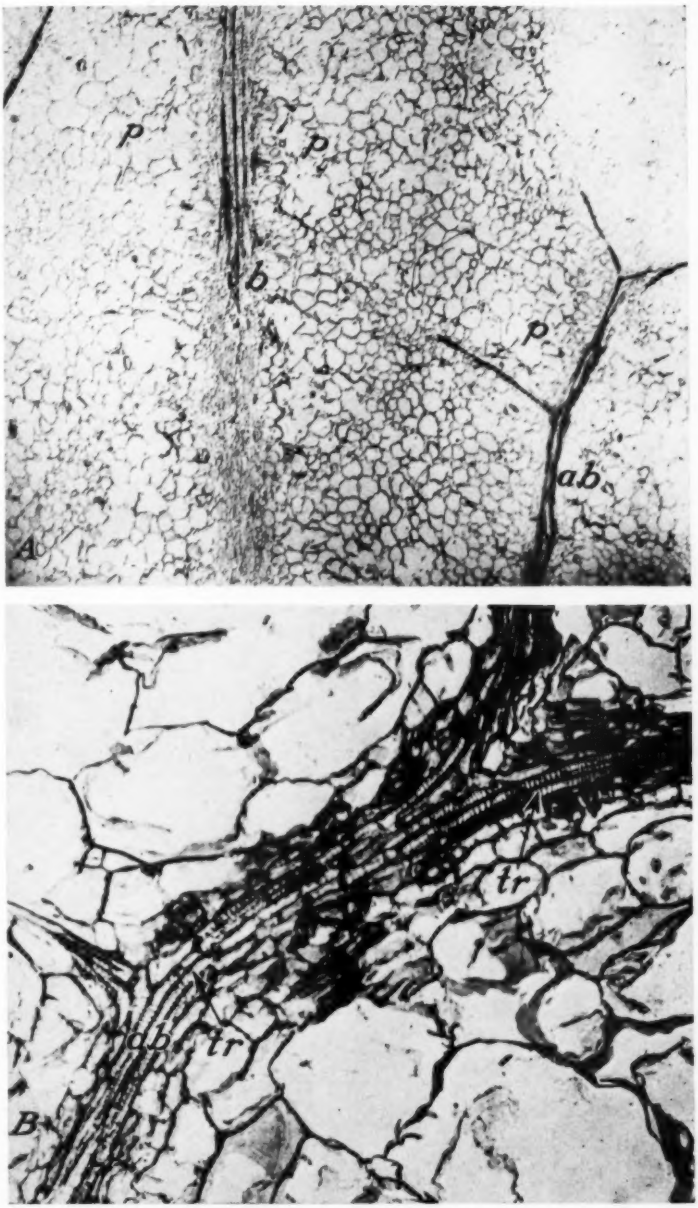
An interesting picture of affected portions of the pericarp and their positions relative to the epidermis and vascular bundles is shown in the transverse section in plate 4, *B*. The epidermis is wavy, being sunken above portions where the underlying parenchyma has collapsed, and unaltered above normal areas and in regions where cavities occur. The dense bands of broken-down cells occur between the outer epidermis and the bundles. In the parts where the epidermis is sunken (pl. 5, *A*), the debris of cell walls and cell contents appears to be forced together; while in the portion where the large cavity appears above the bundle (pl. 5, *B*), fragments of debris have collected around the tracheae and dismembered cell walls are found in the resulting cavity. A similar cavity intermediate between the epidermis and a point near the union of two normal bundles, which are surrounded by unaffected parenchyma, is shown in plate 7, *A*. The accumulation of the dense cellular material between the epidermal layer and the bundles may account for the misconception that the bundles are involved.



A, Seemingly enlarged and discolored bundles with connecting veins and associated tissues as they appear in a cleared wall from a blotchy fruit. The small area (*a*) is shown in plate 7, *A*, and the larger central portion (*b*) is shown in *B*. × 10. *B*, Transverse section of fruit wall shown in *A*, *b*. Note the distribution of the bundles (v_1, v_2, v_3, v_4,) and their relation to the broken-down tissues. The large cavity in the center is through the middle of the dark area shown in *A*. It is the result of rupture during removal of the outer epidermis. See plate 7 for details of the marked areas *a* and *b*. × 10.

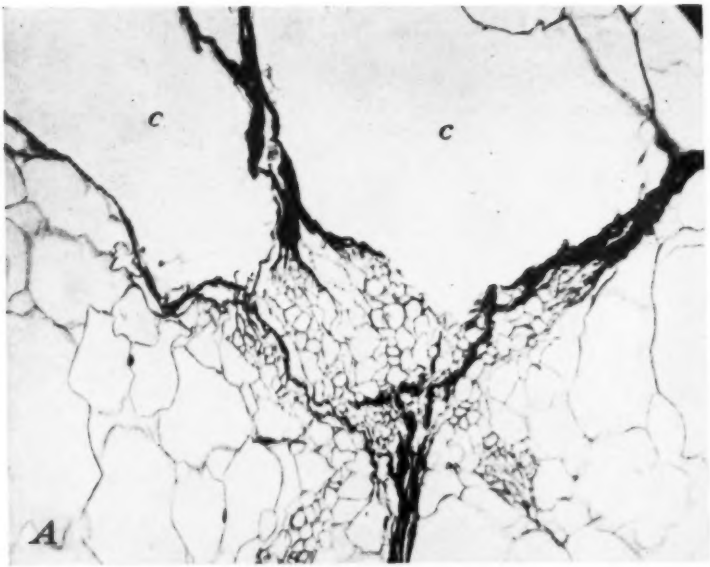


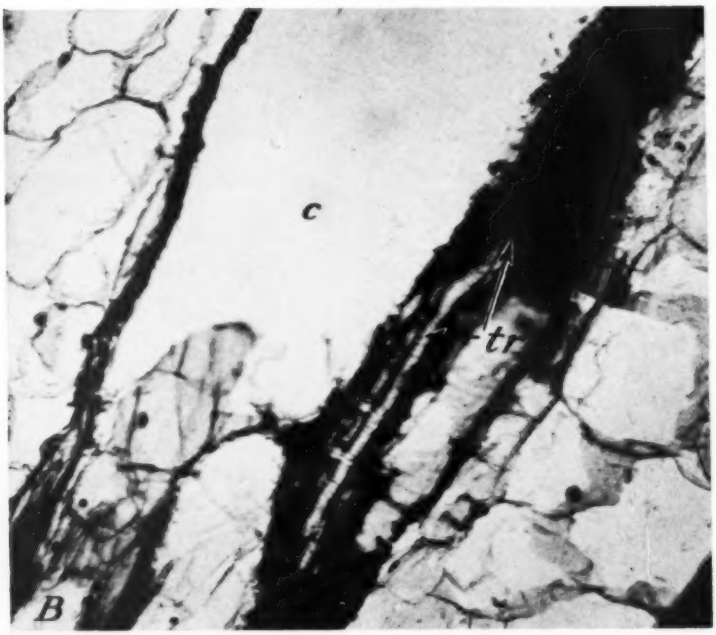
A, Transverse section designated in the upper left, (*a*) of plate 6, *A*, taken in the region of the union of the two bundles. The epidermis (*e*) is shown to the upper left, with a large cavity (*c*) and dark tissues between it and the normal bundles (*vb*). × 50. *B*, Section from area *a* in plate 6, *B*, between the two small bundles (v_1 and v_2). The deteriorated cell material between these bundles has not been compressed as in plate 5, *A*. × 33. *C*, Large cavity shown at *b* in plate 6, *B*, with a margin of discolored and broken-down cell material. Observed with the naked eye or a hand lens, the whole cavity and margin would appear to be an enlarged bundle. See *D* for details. × 33. *D*, Lower portion of cavity shown in *C*. Observe the accumulation of cell debris around the large open tracheae (*tr*) which form the rigid framework for the distorted material. Compare with normal bundle (pl. 2, *E*). × 300.



A, A longitudinal tangential section through the outer wall of a blotchy fruit. Note the broken-down parenchyma (*p*) to either side of the large bundle (*bb*) and between the branches of the anastomosing bundle (*ab*). × 9. *B*, The anastomosing bundle (*ab*) shown in *A* where it seems to be necrotic. At this magnification the spiral thickened tracheae (*tr*) appear normal. × 150.

of cavity shown in *C*. Observe the accumulation of cell debris around the large open tracheae (*tr*) which form the rigid framework for the distorted material. Compare with normal bundle (pl. 2, *E*). × 300.





A, Transverse section through a large bundle with adjacent cavities (*c*). The radiating strands of cell debris appear to have been draped over the strands of tracheary tissue. Note that the parenchyma of the vascular rays found in a normal bundle (pl. 2, *E*) are included in the amassed material. × 250. *B*, Longitudinal view of an adjacent portion of the bundle shown in *A*. The large cavity (*c*) is bordered at the left by distorted cell material, which also covers the tracheae (*tr*) to the right of the cavity. × 250.

Where small, diffused spots appear between the bundles, a different condition exists than in areas where dense accumulations of cellular material occur. The diffused spots are usually made up of broken-down cells which have not been forced together into a dense mass, but are scattered individually throughout the space they formerly occupied (pl. 7, *B*). This scattered arrangement of dismembered cells almost always occurs midway between two bundles (pls. 6, *B* and 7, *B*) and may be considered as a stage intermediate between the normal and the condition where bands of dense material are formed. The bundles associated with the disintegrated parenchyma have been found to be unaffected in almost every case (pls. 7, *B* and 8, *B*). Longitudinal tangential sections of blotchy fruits show this relationship of the bundles to the broken-down parenchyma quite clearly (pl. 8, *A*).

In severe cases of blotchy ripening, large, dense masses of dead cellular material are found to occur in the bundles in such manner that even the tracheae appear affected (pls. 6, *B;* 7, *C* and *D;* and 9, *A* and *B*). Usually, well-defined cavities are present within the necrotic bundle, and the whole mass may give the appearance of the complete distortion of all the elements of the bundle. Nevertheless, under high power one finds the radiating strands of cell debris have been pulled in around the strands of tracheae, which form the framework on which the amassed material is draped. The cells that are actually affected include, for the greater part, the thin-walled parenchyma of the vascular rays, while the tracheae are uninjured and appear to have functioned normally in the areas beyond the blotch (pls. 8, *B* and 9, *A*).

DISCUSSION

The histological observations reported here, as well as data from other investigations of the disorder, substantiate the hypothesis advanced by Seaton [6] (*12*) that blotchy ripening is due primarily to conditions resulting from the withdrawal of water from the fruit during periods of excessive transpiration occurring 2 to 5 days before the fruit ripens, and indicate that deficiencies of potassium and nitrogen, as suggested by Bewley and White (*2*), are probably secondary and occasional. It is evident that blotchy ripening is closely associated with the break-down of the parenchyma near and adjacent to the vascular bundles of the maturing ovary walls. That some physical force is operative in bringing about the conditions antecedent to blotchy ripening is also strongly suggested. Moreover, the very location of the affected tissues indicates that a strong pull, exerted through the vascular system upon the normally large, turgid, thin-walled, delicate parenchyma cells between the bundles and connecting veins of the fleshy layer, results in their rupture and distortion. Indeed, areas similar to that shown in plates 4, *B* and 5, *A* suggest a cleavage of tissue such as might result from loss of turgor, the upper portion being supported by the denser tissues of the outer portion of the pericarp, while the portion below lacks such support and is pulled away.

MacDougal (*8*) has shown by the use of an auxograph that the daily accretion in size of tomato fruits is connected with temperature and

[6] SEATON, H. L. See footnote 5.

water relations. As the temperature of the fruit attached to the plant rose from 12° or 14° to 26° or 28° C., the volume increased to a point where the increased temperature caused an excessive water loss by transpiration which overbalanced the gain by hydration. The midday shrinkage could not be prevented by watering the plants abundantly. His data show that a water deficit may exist in the fruits because of excessive transpiration regardless of the moisture content of the soil. Seaton,[7] using an aniline dye under greenhouse conditions favoring excessive transpiration, has followed the movement of water from the fruits to the transpiring leaves. On bright, warm days in July the dye appeared in the upper leaves in from 1 to 1½ hours after it entered the fruit, while on rainy or cloudy days 4 to 8 hours elapsed before it could be detected in the fruit pedicel.

Similar changes in the direction of the water stream have been advanced as explanations for similar physiological disturbances, such as the blossom-end rot of tomatoes (*3*), the internal decline in lemons (*1*), bitter pit of apples (*7*), the dropping of blossoms in the tomato (*9*), and the shedding of unopened bolls in cotton (*6*).

In the development of some fruits, according to Eames and MacDaniels (*5*), the cells which later form the flesh of the ovary wall are formed during the early development, and development to the mature condition does not involve cell division. The growth consists mostly of the radial enlargement of cells, and in some fruits the formation of intercellular spaces. Thus, in the tomato, ripening involves, histologically, increase in cell size and change in cell shape, the cells becoming turgid with fluid and the walls excessively thin and delicate. According to Sando (*11*), Rosa (*10*), and others, during the ripening processes there is an increase in the percentages of moisture, acids, and sugars, and a decrease in solids, nitrogen, starch, pentosans, crude fiber, and ash. Consequently, the parenchymatous cells of the outer fleshy wall, where the greatest increase in size and the changes in chemical composition occur, become more susceptible to injury as maturity is approached. Since the climatic conditions which usually prevail during the ripening period of the spring crop of greenhouse tomatoes are conducive to excessive transpiration, it is logical to expect the greatest losses from blotchy ripening to occur intermittently during that season.

In the red areas of blotchy fruits the normal ripening and coloring are not retarded, and the processes are completed. However, with the break-down of the parenchymatous cells near and adjacent to the bundles in the blotchy areas, the connections which the outlying cells have for the transfer of elaborated foods and water are severed, and they are deprived of the materials which are essential for normal ripening. Consequently, these areas remain hard and develop the colorless or glassy appearance characteristic of the disorder.

SUMMARY

A histological study of tissues of greenhouse tomatoes affected by blotchy ripening is reported.

This disorder, which affects greenhouse tomatoes that ripen in May, June, and July, is characterized by a failure of areas of the outer fruit wall to develop and color normally. As the fruit approaches maturity, these areas remain hard and green; and as ripening proceeds further,

[7] SEATON, H. L. See footnote 5.

they assume a waxy or glassy appearance. The vascular bundles lying beneath the blotches invariably appear brown and necrotic, and in severe cases cavities may appear adjacent to them. The disorder is restricted to practically mature fruits.

Anatomically, the vascular system of the normal fruit wall consists of large carpellary bundles, their branches, and anastomosing veins. The outer fruit wall is made up of the outer epidermis, the fleshy layer of large parenchyma cells, and the inner epidermis. The bundles are bicollateral, dissected with vascular rays of parenchyma, and with spirally thickened tracheae.

In cleared sections of blotchy fruit walls, large dense areas attendant to the bundles are apparent, while less dense, slightly diffused areas appear between them. Cavities frequently occur in the bundles in severe cases. Histological examination reveals that the discolored tissues are in all cases parenchyma of the fleshy layer and that the bundles are not involved. Bands of discolored cellular material appear between the epidermis and the bundles, which produce the impression that the bundles are affected when viewed through the epidermis. The cavities found in blotchy portions are the result of a collapse of the parenchyma which originally occupied these areas. The diffused spots are composed of dismembered cells from the fleshy layer. Cavities in the bundles result from a collapse of the vascular ray parenchyma which is pulled in around the unaffected tracheae.

The break down of the parenchymatous cells near and adjacent to the bundles in the blotchy areas severs the connections of the outlying cells for a transfer of elaborated materials and water, and normal ripening is inhibited.

The histological observations reported substantiate an earlier hypothesis advanced by the senior author, that blotchy ripening is primarily due to conditions which result from the withdrawal of water from the fruits during periods of excessive transpiration, occurring 2 to 5 days before the fruit ripens.

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THE PROGRESS OF THE DISTRIBUTION OF SALT IN HAM DURING THE CURING PROCESS[1]

By R. C. MILLER, *assistant professor of agricultural and biological chemistry, Department of Agricultural and Biological Chemistry*, and P. T. ZIEGLER, *professor of animal husbandry, Department of Animal Husbandry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Practically all methods of curing pork involve the use of salt as a curing agent. Its main function is as a preservative to prevent or reduce the action of micro-organisms which might cause spoilage in the meat. Aside from this preserving action, the salt contributes greatly to the palatability of the meat. Oversalted pork is undesirable, although it is quite common. The amount of salt present should be sufficient to exert the required preserving action but should not be so excessive as to impair the palatability of the product. In addition the salt should be uniformly distributed throughout the meat to insure uniform quality and palatability.

The process of curing ham involves the application of the curing agent, usually a combination of salt, sugar, and either saltpeter (KNO_3) or potassium nitrite (KNO_2), to the meat by one of two methods, either by dry curing or brine curing. The meat is then allowed to remain in the curing medium for a period of time, which is dependent on the size of the cut and the kind of cure. Subsequently it is soaked in water to remove the excess salt from the outside, smoked, and finally aged or held as long as is desirable to improve its flavor or as is required for practical purposes.

The curing process has as its first objective the penetration of the salt into all parts of the meat rapidly enough to overcome or prevent the deleterious effect of micro-organisms. If the meat is dry-cured an allotted amount of the curing agent is rubbed into it, usually in two or three portions at intervals of several days. Usually the last portion is applied no later than 10 days after the beginning of the cure. In brine curing, which is probably more commonly used, the meat is allowed to remain in a brine or pickle for a period, the length of which should depend on the strength of the pickle used and the temperature at which the cure is conducted. Commercially it is a common practice to pump a strong salt solution into the ham previous to placing it in the pickle proper.

Other things being equal, the effectiveness of salt in preventing bacterial action should depend largely on the temperature at which the curing process is conducted. The optimum temperature is one which is high enough to permit the salt to penetrate as rapidly as possible, and yet low enough to inhibit excessive bacterial action. Temperatures from about 36° to 40° F. seem to fulfill this requirement and are commonly used. Lower temperatures are said to retard the cure,[2] and at higher temperatures spoilage of the meat is liable

[1] Received for publication Sept. 16, 1935; issued March 1936.

[2] MOULTON, C. R. MEAT THROUGH THE MICROSCOPE; APPLICATIONS OF CHEMISTRY AND THE BIOLOGICAL SCIENCES TO SOME PROBLEMS OF THE MEAT-PACKING INDUSTRY. 528 pp., illus. Chicago, 1929. See p. 45.

to occur before a sufficient concentration of salt is obtained to check bacterial action.

The authors have in progress a study of the factors involved in the curing of ham and bacon. The present paper reports their findings in experiments made to determine the degree of distribution of salt in hams cured by different methods, the salt distribution being related to the time allotted to the curing process proper and the time of any subsequent holding or aging.

EXPERIMENTAL PROCEDURE

Fifty-nine hams weighing from 14 to 19 pounds were used for curing. The plan followed was, whenever possible, to put down together, in the curing agent, both the right and left hams from the same hog, and to remove this pair of hams from cure at the same time, then to soak them in water to remove excess salt or curing agent from the outside. One ham was analyzed immediately before being smoked, and the other was smoked and held for a designated number of days, when it was analyzed also. The latter ham gave information as to the effect of holding the ham for an additional period and served as a check on the first.

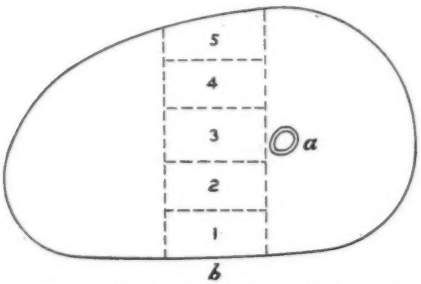

FIGURE 1.—Diagram of method of sampling the hams: *a*, Femur; *b*, skin side.

Each ham was sampled by taking a center cut across it next to the aitch bone.[3] This cut was about 1 inch thick, 3 inches wide, and as long as the thickness of the ham. It was divided into five equal horizontal sections which were separately ground in a food chopper. Each served as a separate sample on which moisture and chlorine were determined. Thus an analysis was obtained on samples representing the center of the ham as well as the two adjacent sections, and the two outside sections. This method of sampling is depicted in figure 1.

The amount of salt present in each section was calculated from the chlorine content as determined by the wet-digestion method as given by Davies.[4] A 5-gram sample of the ground meat was weighed into an Erlenmeyer flask, and to it were added an excess of one-tenth normal silver nitrate, 10 cc of saturated potassium permanganate solution, and 25 cc of concentrated nitric acid. This mixture was boiled vigorously until all solid particles of the meat were completely digested, the average time required being about 20 minutes. Perforated glass beads were used to prevent bumping during the digestion. The solution was then cooled by immersing the flask in cold water. When cool, the excess of silver nitrate was titrated with potassium thiocyanate in the presence of 2 cc of saturated ferric alum and 10 cc

[3] MOULTON, C. R. See p. 112 of reference cited in footnote 2.

[4] DAVIES, W. L. THE DETERMINATION OF CHLORIDES IN DAIRY PRODUCTS AND BIOLOGICAL MATERIAL. Analyst 57: 79–85. 1932.

of acetone. By having a total volume of not more than 100 cc, an easily distinguishable brownish-red end point was obtained without filtering off the white precipitated silver chloride from the yellow solution resulting from the digestion. A blank determination on fresh pork showed it to be so low in chlorine as not to influence the results obtained in this study, in which relatively large quantities of salt were present.

The percentage of salt in the dry matter of each of the five pieces that were subsampled from a ham was computed, and the figures obtained were used to calculate the mean salt content and the standard deviation of the center cut. It should be mentioned that the figures for subsample no. 1 were usually omitted in making the computations, inasmuch as this sample was ordinarily mostly fat and practically always contained less salt than the lean subsamples, presumably due to its lower water content. The coefficient of variability of the salt content was calculated according to the formula

$$cV=\frac{\sigma}{M}\times 100$$

in which cV represents the coefficient of variability.

The value thus obtained is a numerical expression which denotes the extent to which the salt contained in the ham has become distributed or equalized. It is a value which is practically independent of the absolute amount of salt present. Typical figures illustrating the application of this method of expressing the extent of salt equalization are given in table 1, the salt distribution for these two hams being quite different because of different methods of curing.

TABLE 1.—*Data obtained from the five subsamples made from center cuts of two hams cured by different methods and indicating the extent to which the salt was distributed throughout* [1]

Subsample no.—	Salt in ham no.—	
	1	2
	Percent	*Percent*
1 (fat side)	1.05	1.50
2	2.35	8.50
3 (middle piece)	2.89	8.97
4	9.87	8.46
5 (flesh side)	18.89	10.82
Mean salt content of entire center cut	7.01	7.65
Coefficient of variability	90.9	12.1

[1] All analyses are expressed as percentages of the dry matter.

As illustrated by these two hams, the coefficient of variability is high when the distribution of salt in a ham is not uniform, as in the case of ham no. 1, in which the salt is relatively more concentrated in subsamples 4 and 5. Conversely it will be low when the salt content of the several sections sampled is approximately the same, as in ham no. 2. Thus the coefficient of variability is a useful criterion in judging the extent of the distribution of salt and presents a means of comparing different hams in this respect.

It should be pointed out that the mean salt content of the five pieces subsampled from the center cut is not the true mean salt content of the whole ham. This is apparent when it is recognized that the outer portion of the ham (subsamples 5 and 1) represents, on the basis of weight, a different percentage of the whole ham than it represents in relation to the center cut from which it is taken. The same difference exists in a different proportion for the other subsamples of the center cut. As given in table 1 the mean salt content of the entire center cut is 7.01 percent for ham no. 1 while the salt content as determined for the whole ham after grinding it and subsampling was 5.36 percent. The method of center-cut sampling, therefore, is conventional but serves a useful purpose for comparative studies. In addition it serves as an accurate criterion when a consideration of salt penetration in relation to the thickness of the meat is desired, this aspect being equally as important as the relationships involving the weight of the meat.

In table 2 are given data concerning three different brands of standard, first-quality, packer's ham which are taken to be representative of this type of product. These hams were purchased in a local butcher shop and analyzed in the usual manner.

TABLE 2.—*Salt distribution in first-quality commercial hams of three different brands*

Part of ham or sample	Salt in ham no.—		
	1	2	3
	Percent	*Percent*	*Percent*
Middle sample (no. 3)	7.5	12.9	11.2
Outside sample (no. 5)	13.9	15.5	18.1
Mean salt content of entire center cut	11.6	15.8	14.0
Coefficient of variability for center cut	25.8	16.1	24.6

No information is at hand concerning the manner in which these hams were cured or how long they were out of the cure before being sampled. The analysis shows that they contained a considerable amount of salt and that, particularly in the case of hams 1 and 3, the salt content of the outside pieces was somewhat higher than that of the center sample. The coefficients of variability bear out this observation insofar as salt distribution is concerned. A value around 25 would indicate only a fair distribution; thus it is possible that at least two of these hams (nos. 1 and 3) were not held long after curing, or that some other feature of the cure was such as did not permit much equalization of the salt.

SALT DISTRIBUTION IN DRY-CURED HAMS

In table 3 are given data for hams cured under experimental conditions. Both groups were dry cured with salt and saltpeter. The hams having the same curing period were from the same hog.

The curing agent was applied at the rate of 1 ounce per pound to the skinback hams and 2 ounces per pound to the regular hams, a portion as large as could be immediately taken up by the meat being applied in one rubbing, the remainder being sprinkled on the meat. The curing was done in wooden boxes from which the liquor exuding from the hams was allowed to drain, and in a refrigerated room where

the temperature was controlled to about 37° F. Pairs of hams were removed from the cure at the same time, one ham from each pair being immediately analyzed, and the other being held for an additional period, as indicated in the table, during which time it was smoked and then analyzed. The hams were held in a refrigerated room previous to smoking, and at room temperature after smoking.

TABLE 3.—*Salt distribution while curing and after holding in hams dry-cured for various periods with salt and saltpeter at 37° F.*

Ham no.[1]	Regular hams			Ham no.[1]	Skinback hams		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
7^3	20	0	123.8	3^4	28	0	92.8
1^3	35	0	100.9	10^4	35	0	98.5
5^3	42	0	81.9	2^4	42	0	76.7
10^3	20	30	52.8	5^4	28	32	14.0
2^3	35	30	25.8	1^4	35	32	36.0
3^3	42	30	34.4	6^4	42	32	20.0

[1] The superior figures are for laboratory records.

The results indicate that with both methods of curing the equalization of salt was progressing throughout the whole period of observation, which was from 20 to 74 days. However, the coefficients of variability are somewhat lower in five out of the six cases for the skinback hams, indicating a somewhat more rapid cure. The slower cure with the regular hams may be attributed to the heavier fat covering on the hams, this layer of fat retarding salt penetration.

It is apparent that the type of results presented in this paper show considerable variation because of the small number of hams that have been tested. Therefore, the results are of limited value in making close comparisons unless large numbers of observations are made, but are useful for the present purpose in indicating the general progress of salt penetration and equalization.

In table 4 are presented data for hams dry-cured with salt, brown sugar, and saltpeter, using 1 ounce per pound of meat, applied in three rubbings. One series of hams was kept during the cure in an outside brick building which was unheated, and with the temperature uncontrolled. The conditions simulated those that would be encountered in farm practice. The other series was cured under controlled conditions.

Salt alone or in combination with saltpeter is commonly used in farm practice for curing meats, but it is generally conceded that a product of better quality is obtained if the curing agent contains some sugar. Unpublished experimental work by the authors has shown that a mixture of 8 pounds of salt, 3 pounds of brown sugar, and 3 ounces of saltpeter is a satisfactory curing mixture.

Data for an odd ham are presented in the controlled series, its mate having been erroneously cut before the termination of the cure; also hams 2^A and R^2 were not from the same hog but were practically identical otherwise.

TABLE 4.—*Salt distribution, while curing and after holding, in hams dry-cured for various periods with salt, brown sugar, and saltpeter, under uncontrolled temperature conditions and at 37° C.*

Ham no.[1]	Cured with salt, brown sugar, and saltpeter, curing temperature uncontrolled. (40°–60° F.)			Ham no.	Cured with salt, brown sugar, and saltpeter at 37° F.		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
5^{S}	28	0	67.7	2^{A}	21	0	108.8
1^{S}	35	0	104.1	3^{S}	26	0	111.9
3^{S}	42	0	57.3	6	28	0	120.8
10^{S}	28	32	18.5	10^{S}	33	0	55.3
1^{S}	35	32	12.8	7	35	0	70.7
6^{S}	42	32	2.9	2^{S}	40	0	59.8
				5	42	0	48.7
				R[2]	20	30	32.3
				6^{S}	26	30	12.7
				1	28	30	15.9
				2	35	30	12.2
				1^{S}	40	30	8.4
				3	42	30	11.0

[1] The superior numbers and letters indicate skinback hams. The other hams were regular.

In all the hams that were cured for 26 days or more and then held for 30 days, the distribution of salt, as judged by the coefficients of variability, was uniformly good. It appears that salt equalization was progressing at a rather uniform rate during both the curing and the holding periods and would be satisfactory after 50 to 60 days. Also there is some indication that the distribution of salt in these sugar-cured hams, as related to time, is somewhat better than in the case of hams cured with salt and saltpeter under controlled conditions. A comparison of hams that were cured for approximately the same periods and then held for a period after cure, shows that the coefficients of variability are lower in all cases for the sugar-cured hams.

SALT DISTRIBUTION IN BRINE-CURED HAMS

The results obtained with two series of brine-cured hams are given in table 5. The brine was made up of 8 pounds of salt, 3 pounds of brown sugar, and 3 ounces of saltpeter, dissolved in 4 gallons of water and used at the rate of 4 gallons per 100 pounds of meat. The series of hams, for which data are given in table 5, were in part cured under controlled temperature conditions at about 37° F., and in part under uncontrolled temperature conditions in the smokehouse previously described.

In the cases where comparisons are possible, it does not appear that there is much difference in salt equalization in the hams cured under either uncontrolled or controlled conditions.

The coefficients of variability of the salt of the hams cured in brine under controlled conditions are about the same for all the hams analyzed after 25 to 42 days in the brine. The figures for hams cured from 25 to 30 days appear to be lower than for those dry-cured for approximately the same period. It might be expected that such might be true since, in dry curing, all the salt which is ultimately

distributed throughout the ham is rubbed into the surface within the first few days after the beginning of the cure. From then on the salt is penetrating further into the ham and is becoming distributed throughout it, the concentration of salt gradually being diminished in the outside portion. In brine curing, salt is being continually taken up by the meat from the brine until the cure in the brine is terminated. It would appear from the coefficients of variability, that in brine-cured hams the concentration of salt in the outside portion in relation to the concentration in the inside is never as great as it is in the early stages of dry curing. Because of the continued adsorption of salt during the brine cure, equalization of the salt may not be as rapid as in dry curing during the cure proper. The data show that, in the case of brine curing, most of the equalization occurs during the holding period following the cure.

TABLE 5.—*Salt distribution in hams brine-cured for various periods, both at about 37° F. and under uncontrolled temperature conditions, while curing and after holding*

Ham no.[1]	Cured at about 37° F.			Ham no.	Cured under uncontrolled temperature conditions (40°–60° F.)		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
1^B	25	0	84.7	0^A	28	0	101.3
7	28	0	83.8	3^A	35	0	72.0
5^E	30	0	81.0	2^C	35	0	65.3
X^E	34	0	80.5	X^C	35	6	65.1
2	35	0	90.9	4^A	42	0	54.6
5	38	0	84.6	1^C	35	30	31.8
1	42	0	73.6	2^A	28	61	10.6
0^B	42	6	73.6	1^A	35	54	9.5
0	28	30	3.2	5^A	42	61	13.1
0^E	34	30	33.2				
2^B	35	32	17.1				
3^B	42	30	13.6				
3	35	64	17.8				
6	42	60	6.4				

[1] The superior letters are for laboratory records.

No mention has been made of the influence of smoking the meat on the salt penetration. It might be expected that exposure to the higher temperature of the smokehouse would hasten the equalization of salt, but as yet the present study has not particularly concerned this point and such evidence as has been obtained is inconclusive, although there is some indication that smoking slightly speeds up salt equalization.

With respect to the ultimate distribution of salt, the dry-curing and brine-curing processes are essentially the same. However, they must differ in fundamental details. Experiments are cited by Callow [5] in which small pieces of pork muscle were immersed in concentrated solutions of salt. At first, water diffused from the muscle to the solution, the rate of diffusion depending on the concentration of the salt solution. Finally, the water began to diffuse back from the strong solution of salt to the muscle. Apparently this phenomenon

[5] CALLOW, E. H. THEORY OF CURING. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1932: 101–102. 1933.

does not occur except in the presence of proteins, there being formed a salt-protein complex which has an osmotic pressure greater than that of the surrounding solution.[6] Since the salt-protein complex is hypertonic to the solution, water diffuses back into the protein-containing system.

Callow [7] has also shown that the uptake of salt from solutions of different strengths is continuous and is dependent on the strength of the brine used, more salt being taken up from the stronger solutions.

These findings agree with those of practice, the increase in weight of brine-cured hams due to uptake of water being commonly recognized. The results herein presented show, however, that under ordinary conditions the salt in a ham is not uniformly distributed for some length of time. It is doubtful, with a heterogeneous-phase composition with respect to salt and tissue constituents, whether equilibria with respect to salt and water uptake are reached in ordinary curing practice. Methods of curing, therefore, must be controlled procedures which produce the desired effect.

SUMMARY

A statistical method is presented, by means of which it is possible to express mathematically the extent of the distribution of salt in ham at various stages in the process of curing.

Results are given for 59 hams, variously cured.

The salt in hams cured by various methods did not become well distributed throughout the meat until approximately 60 days after the beginning of the various cures. Salt equalization begins in dry-cured hams as soon as the last portion of curing mixture is applied to the meat. In brine curing, salt equalization is accompanied by absorption of salt from the brine until the meat is removed from it, thereafter salt equalization alone takes place.

In all the curing methods used, the aging of hams for 30 days after their removal from the cure permitted further equalization of the salt and gave a product of more uniform salt content.

[6] ADAIR, G. S. A THEORY OF PARTIAL OSMOTIC PRESSURES AND MEMBRANE EQUILIBRIA, WITH SPECIAL REFERENCE TO THE APPLICATION OF DALTON'S LAW TO HÆMOGLOBIN SOLUTIONS IN THE PRESENCE OF SALTS. Roy. Soc. [London], Proc. A 120: 573-603, illus. 1928.

[7] CALLOW, E. H. SECTION C.—PIG PRODUCTS. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1929: 67-73, illus. 1930. See p. 69.

THE EFFECT OF FREEZING ON THE PHYSICAL AND MISCROSCOPIC CHARACTER OF GELS OF CORN AND WHEAT STARCHES [1]

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INTRODUCTION

The number of changes both in the gross and in the microscopic appearance of starch gels brought about by freezing is so great that it suggests many lines of study in connection with the structure of starch granules. It is believed that the facts established in this investigation may be applicable to the problem of low-temperature preservation of food and to that of water removal from food materials containing gelatinized starch. The differences found between corn and wheat starches may be of value in determining the kind of starch for practical use.

METHODS

SOURCE AND PREPARATION OF STARCHES

Starches of both corn (*Zea mays*) and wheat (*Triticum vulgare*) were prepared by methods designed to leave them as nearly in their native state as possible, while at the same time freeing them from other constituents of the kernel. Three varieties of corn, Reid Yellow Dent, Krug Yellow, and Champion White Pearl, were used as sources of starch. So little difference between them was found that photomicrographs for the Reid corn only are shown.

The corn was soaked overnight in cold water and ground several times to medium-fine particles. Its starch was washed out and separated from the other material of the kernel, first on a 60-mesh screen, then on fine bolting cloth. The starch suspension was centrifuged and washed in turn with 2-percent sodium chloride, water, alcohol, and ether. Layers of gluten at the top of the starch layer in the centrifuge bottles were scraped off. This starch was air-dried for use.

Wheat starch was prepared by the method previously reported by Woodruff and Webber (*9*)[2] from 1934 Fulhio wheat flour milled especially by a commercial soft-wheat mill for another experiment station project. Slight physical differences between starches separated from bleached and unbleached flours were found, but microscopic differences were negligible and photomicrographs only of unbleached flour starches are shown.

FREEZING OF STARCH GELS

Starch gels were made by heating suspensions containing 5 percent of starch by weight in a boiling water bath. The starch was first moistened with a little cold water in a conical flask. The boiling water was added and the flask swirled until a thermometer in the

[1] Received for publication Aug. 5, 1935; issued March, 1936. A brief paper covering part of these findings was read before the food and agricultural division of the American Chemical Society in New York City in April 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 237.

paste reached the temperatures 70°, 75°, 80°, 85°, 90°, and 95° C. The pastes were poured immediately into molds and allowed to cool and set to a gel overnight. One set of molds was placed in the freezing unit of an electric refrigerator where the lowest temperature reached by them was −2° to −3°. After 5 or more hours they were removed from the freezer, allowed to thaw at room temperature, and examined microscopically. Another set of molds was packed in solid carbon dioxide and left for 1 hour, after which they were removed and allowed to thaw at room temperature. Samples were also frozen in liquid air but these were not different from the ones frozen in solid carbon dioxide.

MICROSCOPIC EXAMINATION

Specimens were removed from the gels with a knife tip, special care being taken to avoid cutting the shreds in the frozen ones. The specimens were then mounted in water for microscopic examination. Photomicrographs were made with ordinary light at a magnification of 900 times. Polarized light was also used for the examination of each specimen between crossed nicols.

RESULTS

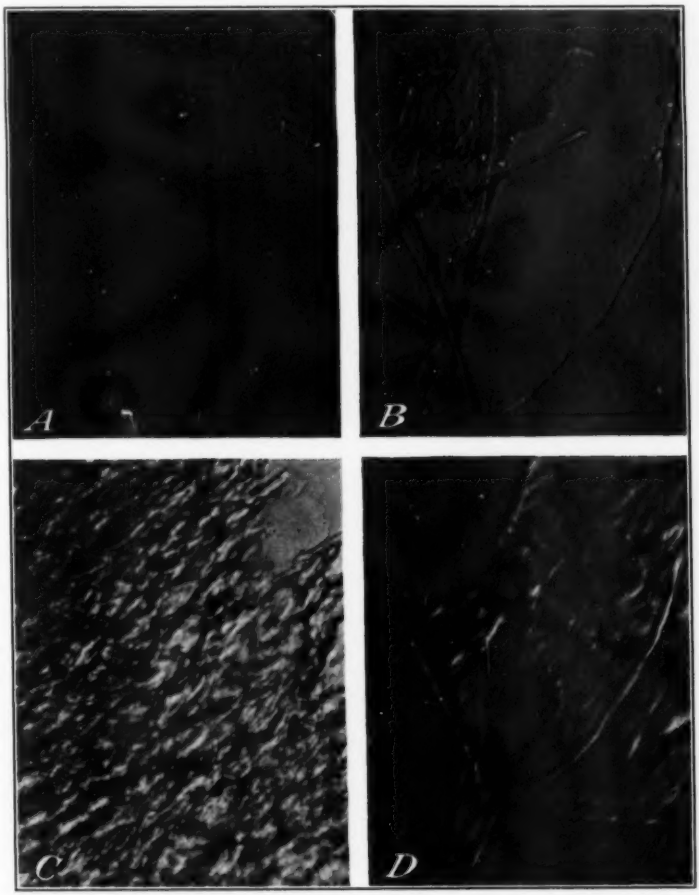
PHYSICAL CHANGES IN GELS CAUSED BY FREEZING

The following discussion is for the most part confined to the behavior of gels made by heating starch suspensions to 95° C. The cornstarch made a well-formed gel even at 75° or 80°, but wheat starch did not until it was heated to a temperature of 95°, for at the lower temperatures some starch granules remained unswollen.

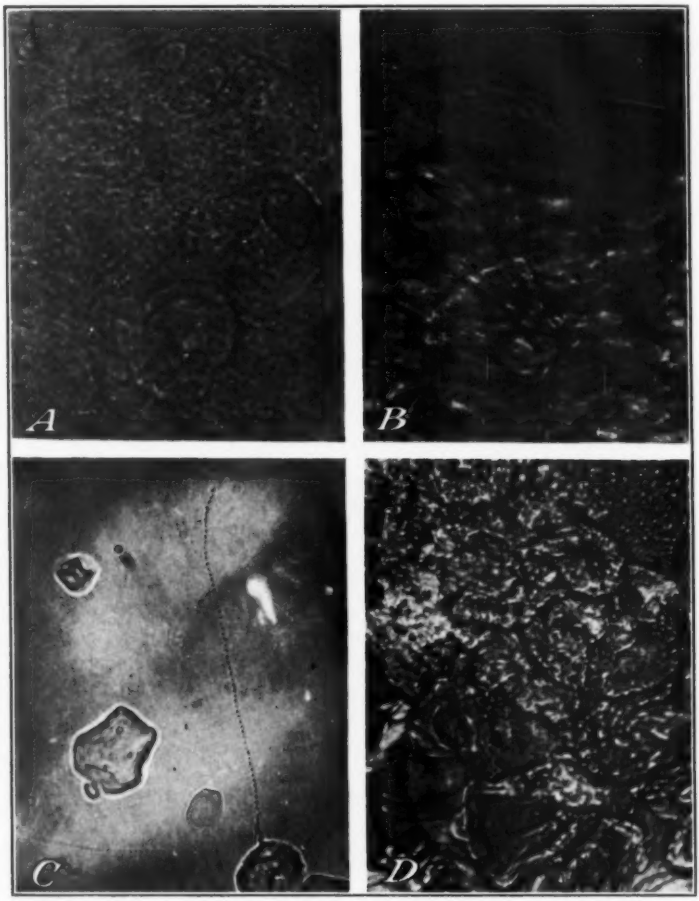
The cornstarch gel after being frozen slowly where it reached a temperature no lower than −2° to −3° C. became coarsely fibrous with some radial orientation of the fibers. It was spongelike in that water could be pressed from it without its losing shape and it greedily absorbed water again. It likewise absorbed water solutions of either iodine or of dyes. The original gel, on the other hand, was not penetrated by an iodine solution dropped on its surface; moreover, it was bluish white, pasty, uniform in consistency so far as the unaided eye could detect, and did not give up water when pressed. It dried in air to a horny film which could be remoistened only with difficulty. The sponge of the frozen gel, pressed free from water, dried in air, giving a porous, fibrous, friable substance which absorbed water as easily when dry as it had when moist. The starch thus retrograded by freezing could be warmed with water and dispersed sufficiently to make a gel again similar in appearance to the original one, though less uniform in consistency. The freezing and dispersing were repeated many times without noticeable loss of power to form a gel. Even in gelatinized suspensions of 1-percent concentration instead of the 5 percent used, the fibrous arrangement could be seen.

Frozen in solid carbon dioxide, the gel changed in gross appearance much less; it retained most of its pasty appearance and though it gave way when pressed, no water came from it. It dried to a horny film very like the original gel.

Wheat-starch gels frozen in the refrigerator were fibrous like those of cornstarch, but the sponge formed was not so tough and water could not be squeezed out so readily. They differed decidedly from



Photomicrographs of frozen cornstarch gels. *A*, Gel before freezing; *B*, 1-percent suspension, frozen at −2° to −3° C.; *C*, gel frozen in solid carbon dioxide; *D*, gel frozen at −2° to −3°. All were gelatinized at 95°; all except *B* contained 5 percent starch. × 900.



Photomicrographs of frozen starch gels and of optically active particles in starch. *A*, Wheat-starch gel before freezing; *B*, wheat-starch gel frozen at −2° to −3° C.; *C*, cornstarch gelatinized at 75°; *D*, wheat starch gelatinized at 85° and then frozen in solid carbon dioxide. Note optically active particles in *C* and *D*. × 900.

the frozen cornstarch gels in that they dried to a horny mass which did not readily reabsorb water. This point was noted by Holton,[3] who worked on a similar problem in this laboratory. Gels frozen in solid carbon dioxide were again more like the original ones and lacked the fibrous structure produced at −2° to −3° C.

MICROSCOPIC APPEARANCE OF FROZEN GELS

Freezing effected changes as pronounced in the microscopic appearance of gels as in the gross. Evidence of these changes is presented in plates 1 and 2.

It was not easy to show the structure of a starch gel microscopically. The swollen granules were very transparent after being heated to 95° C., were packed against each other, and their details were indistinct (pl. 1, *A*). The remarkable change in appearance brought about in cornstarch gels by freezing is shown in plate 1, *C* and *D*, which presents photomicrographs of a gel frozen in solid carbon dioxide and of one placed in the freezing unit of a refrigerator where it reached a temperature of only −2° to −3°. The very heavy veins in the latter seemed to correspond to the fibrous structure noticed on gross examination. Freezing more quickly at the lower temperature produced less change in microscopic as well as in gross appearance, though veining of a light nature was apparent. Plate 1, *B*, shows that even in a 1-percent suspension, gelatinized starch was profoundly altered by freezing; long fibers are apparent there.

Wheat starch became even more indistinct in outline than cornstarch when gelatinized at a temperature of 95° C. Plate 2, *A* and *B*, shows the wheat starch as it appeared in the original gel and after being frozen at −2° to −3°. Definite lines of reorientation are visible in the latter though the heavy veins of frozen cornstarch gel are absent.

Starch grains lost their anisotropy as soon as they swelled slightly, Very small, oval-round particles which transmitted polarized light could be seen, however, after birefringence had disappeared. These particles were somewhat visible at all temperatures of gelatinization though they were more plentiful and more distinct at temperatures under 95° C. In many cases where particles occurred singly they showed a Brownian movement. A few such particles can be seen in the cornstarch gel previously heated to 95°, shown in plate 1, *A*, and a suggestion of their presence is to be noted also in the wheat-starch gel gelatinized also at 95°, of plate 2, *A*, though in the latter case the camera was focused on the outer boundaries of starch granules, hence these small particles were not in focus. Plate 2, *C* and *D*, shows these oval-round particles in corn and wheat starches which had been heated to temperatures of only 75° and 85° respectively. When slight pressure was exerted on the cover glass during the examination of starch gelatinized at temperatures of 70° or somewhat above, such streams of these particles as are shown in plate 2, *C*, were often observed. Short chains of them under or on the surface of the swollen starch granules were noted more frequently than the long streamers just mentioned. The wheat starch of plate 2, *D*, shows a surface on which such particles are visible in great numbers.

[3] HOLTON, M. THE EFFECT OF RETROGRADATION ON THE SUBSEQUENT GELATION OF WHEAT STARCH. Unpublished master's thesis. Univ. Ill. 1934.

The veinlike structure appearing in the photomicrographs of frozen gels, particularly of those frozen at −2° to −3° C., also transmitted polarized light. Likewise did dried shreds of frozen starch gels mounted without water (not shown in the plates).

DISCUSSION

Only a few investigators seem to have mentioned the appearance of frozen starch gels and no photomicrographs of them have come to the writers' attention. Samec (*8*) dialyzed gelatinized and frozen potato starch for soluble starch on which he made determinations of phosphorus. Baldwin (*1*) used freezing as a means of separating alpha and beta amyloses of potato starch. Reilly, O'Donovan, and Murphy (*7*) centrifuged the clear liquid from the fibers formed by freezing a 5-percent suspension of gelatinized potato starch and recovered an amylose on which to make molecular weight determinations.

The literature contains several references to what might correspond to the oval-round particles pointed out in the accompanying plates. Several investigators, Samec (*8*), Hanson and Katz (*3*), and Hess and Rabinowitsch (*4*), in recent years have discussed crystallin or micellar structure in line with the very early conceptions of starch constitution. Hanson and Katz reproduced with drawings the arrangement of blocklike units in potato, arrowroot, and wheat starches, made suitable for observation by swelling with calcium nitrate. Hess and Rabinowitsch photographed by cinematograph the material issuing from a punctured potato-starch granule. The irregular mass consisted of particles having a Brownian movement. Linsbauer (*6*) has followed the gelatinization changes in potato starch with a hot-stage microscope; his drawings, however, do not include such particles as are shown in this paper. There is at least a suggestion in the resemblance in appearance of the particles of the accompanying photomicrographs to the cellulose particles found by Farr and Eckerson (*2*) in cotton fibers, that the particles seen here in starch have something fundamental to do with the structure of the starch granule.

The observation that gels showed a greater change in both gross and microscopic appearance at −2° to −3° C. than they did at much lower temperatures, was in accord with a result of Katz' (*5*) work on bread staling. The staling process which is concerned with changes in starch was found by him to pass through a maximum value at −2° to −3°, when bread was stored at temperatures varying over a wide range.

A study of the photomicrographs made in the course of this investigation lead the writers to believe that the reticulation of the starch gels, frozen at −2° to −3° C. and later thawed, may possibly have resulted from the association of micelles or aggregates which in turn had formed when the dehydration of the swollen starch granules by ice-crystal formation permitted the molecules to be drawn closer together through secondary valence forces. The explanation offered for lesser change at the still lower temperatures of solid carbon dioxide and liquid air is that less injury occurred with rapid formation of smaller ice crystals, and the temperature was, moreover, too low easily to permit of the physical changes of reorientation. Indications of this lie in the fact that if a gel, previously frozen in solid carbon

dioxide, was kept for many hours in the freezing unit of the electric refrigerator while its temperature rose it would assume practically the same microscopic characteristics as the gel that had been all the time in the unit at −2° to −3°.

SUMMARY

Corn and wheat starches were gelatinized at temperatures ranging from 70° to 95° C. and gels of 5 percent concentration were then frozen at −2° to −3° and also at the temperature of solid carbon dioxide. After thawing they were examined for physical and microscopic changes. In gross appearance the frozen gels were very different from the original ones. At −2°, where the effect of freezing was greatest, the gel became like a fibrous sponge from which most of the water could be pressed. Other changes in physical properties also were noted.

Photomicrographs are shown of corn and wheat starches which had been first gelatinized, then frozen. Veined areas appeared in gels after slow freezing at −2° to −3° C. Rapid freezing in solid carbon dioxide produced fewer changes both in the gross and in the microscopic appearance of gels than did slow freezing at −2° to −3°. The veinings in the frozen gels were optically anisotropic as were also small oval-round particles seen constantly at different stages of swelling.

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